Effets du changement climatique sur les écosystèmes littoraux de la Mer Méditerranée Nord-occidentale: étude de la relation entre les conditions de température et la réponse biologique pendant les événements de mortalité massive

THÈSE DE DOCTORAT DE L'UNIVERSITÉ DE LA MÉDITERRANÉE

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présentée par
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Effets du changement climatique sur les écosystèmes littoraux de la Mer Méditerranée Nord-occidentale: étude de la relation entre les conditions de température et la réponse biologique pendant les événements de mortalité massive.

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Trempe dans l'encre bleue du Golfe du Lion,
Trempe, trempe ta plume, ô mon vieux tabellion,
Et de ta plus belle écriture,
Note ce qu'il faudrait qu'il advînt de mon corps
Lorsque mon âme et lui ne seront plus d'accord,
Que sur un seul point: la rupture….

….Juste au bord de la mer, à deux pas des flots bleus,
Creusez si c'est possible un petit trou moelleux,
Une bonne petite niche,
Auprès de mes amis d'enfance, les dauphins,
Le long de cette grève où le sable est si fin,
Sur la plage de la corniche….

C'est une plage où même à ses moments furieux,
Neptune ne se prend jamais trop au sérieux,
Où quand un bateau fait naufrage,
Le capitaine crie: «Je suis le maître à bord!
Sauve qui peut, le vin et le pastis d'abord,
Chacun sa bonbonne et courage.»….

….Est-ce trop demander: sur mon petit lopin,
Plantez, je vous en prie, une espèce de pin,
Pin parasol de préférence,
Qui saura prémunir contre l'insolation
Les bons amis venus faire sur ma concession
D'affectueuses révérences….

….Pauvres rois pharaons, pauvre Napoléon,
Pauvres grands disparus gisant au Panthéon,
Pauvres cendres de conséquence,
Vous envierez un peu l'éternel estivant,
Qui fait du pédalo sur la vague en rêvant,
Qui passe sa mort en vacances….

George Brassens, Supplique Pour Être Enterré À La Plage De Sète, 1966

Qué maravilla estar admirando a los peces en su pecera y de golpe verlos pasar al aire libre, irse como palomas (Julio Cortázar, Rayuela, 1963).

A mis padres

Les photos de ce manuscrit sont d'Eduardo Arias Galan, Olivier Bianchimani, Joaquim Garrabou, Mari Carmen Pineda, Frédérique Zuberer et Carolina Crisci.

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Résumé

Le changement climatique touche l'ensemble des écosystèmes marins de la planète. Au cours de ces dernières années, les communautés benthiques des fonds rocheux de Méditerranée nord-occidentale (MNO) ont été impactées par des événements de mortalité massive (EMM) d'une étendue et d'une intensité sans précédents. Ces EMM, associés à des anomalies thermiques positives de la colonne d'eau, se caractérisent notamment par une intensité variable en fonction des localités et des échelles spatiales considérées (régions, profondeurs ou populations d'une même région et profondeur). Considérant la tendance au réchauffement de la MNO, il est probable que de nouveaux EMM se produiront. Progresser dans la connaissance de la relation entre la température (T) et la mortalité ainsi que dans les causes de la variabilité observée dans la réponse biologique est indispensable afin d'anticiper les effets des futures anomalies thermiques et de développer des stratégies de gestion et de conservation efficaces dans un contexte de changement climatique.

Dans la première partie de cette thèse (Chapitre 2), l'analyse de séries de T à haute résolution (horaire) à partir de nouveaux descripteurs statistiques, a permis la caractérisation du régime thermique de quatre régions de la MNO et ce, pour différentes années (période de 1999 a 2006) et profondeurs (10 et 25 m) avec et sans EMM. Les différences du régime thermique mises en évidence entre ces régions, profondeurs et années ont expliqué la variabilité des niveaux d'affectation observée dans les populations de *Paramuricea clavata*, espèce modèle de ce travail. Parallèlement, la combinaison de donnés d'expériences de thermotolérance disponibles pour différentes espèces benthiques de fonds rocheux de la MNO avec les conditions de stress thermique maximal observées sur le terrain a permis de proposer des scénarios de risque de mortalité dans les quatre régions d'étude. Étant donné que dans cette partie de la thèse, les populations de *P. clavata* au sein de chaque région, année et profondeur ont été soumises à différents types d'anomalies thermiques pendant les EMM, il n'a pas été possible d'étudier l'importance d'autres facteurs tels que l'acclimatation et/ou l'adaptation au régime thermique en tant que conditionneurs de la réponse à la T. Pour étudier la réponse à la température de populations de *P. clavata* qui habitent des régions à régimes thermiques différents, nous avons réalisé des expériences de thermotolerance à partir de populations provenant de trois régions et de deux profondeurs caractérisées par des régimes thermiques contrastés (Chapitre 3). Nous avons d'abord pu établir que 25º C est une température critique pour *P. clavata,* ce qui a suggéré que les populations peu profondes vivent près de leur limite supérieure de thermotolerance. Ensuite, la plus grande variabilité de réponse au stress thermique ayant été observée entre populations de la même région et profondeur et non entre populations de régions ou de profondeurs différentes, nous suggérons que des processus d'adaptation à des conditions locales seraient susceptibles de moduler la réponse au stress des populations de *P. clavata*. Enfin, la présence d'une proportion relativement élevée de colonies résistantes dans la grande majorité des populations étudiées, après plusieurs jours d'exposition à différentes températures pourrait indiquer une aptitude à s'acclimater et/ou à s'adapter à un changement du régime thermique. Dans le Chapitre 4, nous présentons les techniques d'*Apprentissage Statistique* et analysent leur utilité pour traiter des données écologiques. Dans le Chapitre 5, nous appliquons certaines de ces techniques afin de développer des instruments de prédiction des EMM à partir de variables de T. Ces techniques ont démontré d'être utiles pour prévoir les EMM puisqu'associées à des taux d'erreur relativement faibles.

Ce travail de thèse apporte donc un nouvel éclairage sur le rôle de la température dans les EMM en MNO et a permis, dans le même temps, le développement d'un outil de prédiction de ces événements. Nos résultats soulignent notamment l'importance de l'acquisition de séries de températures à haute résolution et le rôle crucial d'une analyse combinée aux données biologiques de terrain et de laboratoire en considérant un échèle régionale. L'intégration de ce travail à d'autres études telles que celles menées sur la génétique de populations

ou sur les communautés bactériennes associées aux différentes espèces impactées devrait permettre une amélioration significative de la gestion et de la conservation des communautés benthiques de fonds rocheux de Méditerranée nord-occidentale, *hotspot* de biodiversité.

Mots clé: séries de températures à haute résolution; anomalies thermiques positives; communautés benthiques côtiers de substrats rocheux; populations de *Paramuricea clavata;* Méditerranée nord-occidentale; événements de mortalité massive; changement climatique; thermotolerance; acclimatation/adaptation; *Apprentissage Statistique*; données écologiques; prédiction.

Abstract

Climate change is an emerging threat affecting marine ecosystems worldwide. In recent years, mass mortality events (MME) of unprecedented extension and severity were observed in the coastal rocky benthic communities of the North-Western Mediterranean (NWM) Sea. The registered MMEs were concomitant with thermal anomalies in the water column. A trait of these events was the great variability in mortality pattern observed at all spatial scale considered (between regions and depths and between populations of same regions and depths). Since a warming trend was addressed for the NWM region, new MME are likely to occur in the future. For this reason, deepening the knowledge on temperature (T)-mortality relationship as well as on the causes of the observed variability in the biological response, will allow to anticipate the occurrence and effects of future thermal anomalies and contribute to develop management and conservations strategies in the face of climate change.

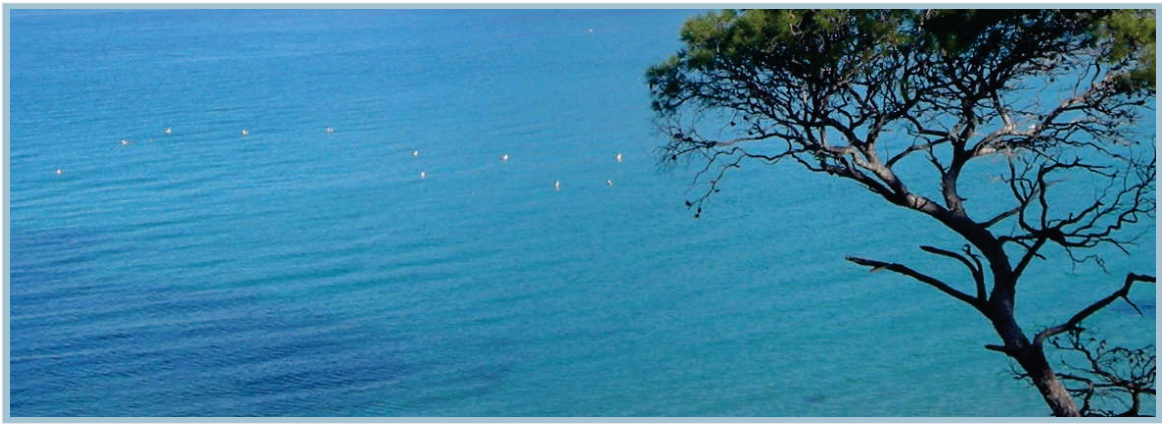
In the first part of this thesis (Chapter 2) a detailed analysis of high resolution (hourly) T series, using a new panel of statistics allowed the characterization of the thermal regime of four regions of the MWM considering several years (1999-2006 period) and two depths (10 and 25 m) with and without MME. Differences in the T regimes allowed explaining inter-regional, inter-depth and inter-annual differences in mortality impacts of *Paramuricea clavata* populations (considered as a model species). Furthermore, the combination of available thermotolerance experiments data of different NWM rocky benthic species with maximal stress T conditions observed in the field allowed determining differential risk of mass mortality across the four study regions. Because in this part of thesis, *P clavata* populations within each region, year and depth were subjected to different type of T anomalies during MME, it was not possible to study the presence of factors such as acclimatization or adaptation to local thermal regime in conditioning the response to abnormal T. To study the response to T of populations inhabiting regions with different thermal regimes, thermotolerance experiments were conducted with *P. clavata* populations dwelling in three regions and two depths of the NWM that present contrasted thermal regimes (Chapter 3). The experiments results indicated in the first place that 25^{o}C is a critical T to *P. clavata* which might be suggesting that shallow populations of the species are living near their upper thermotolerance limit. A second important result was that greatest variability in the response to temperature was fond at the inter-population level (populations from the same region and depth) and not between populations from different regions and depths The great variability at the inter-population level could be indicating that adaptative processes to local conditions can modulate the response to stress of *P. clavata* populations. Finally, the presence of relatively high proportion of resistant colonies in most of the studied populations after several days of exposition to different Ts could be indicating a potential capacity to acclimatize and/or adapt to changes in the thermal regime. In Chapter 4, *Machine Learning* techniques were introduced and their suitability to treat ecological data was analyzed. In Chapter 5 we applied some of these techniques to generate a predictive tool of MME from temperature variables. These techniques were useful to predict MME giving relative low error rates.

The results of this thesis give new insights in the role of temperature in the MME of the NWM as well as a predictive tool of these events. The results suggested for the importance of the acquisition of high resolution temperature series as well for the importance of a combined analysis of field and experimental biological data considering a regional scale. The integration of this information with results of other studies such as those of population genetics and of the bacterial communities associated with affected species, are of great importance to improve management and conservation of the coastal rocky community of the *hotspot* of biodiversity that is the NW-Mediterranean.

Key words: high resolution temperature series; positive thermal anomalies; coastal rocky benthic communities; *Paramuricea clavata* populations; NW Mediterranean; mass mortality events; climate change; thermotolerance; acclimatization/adaptation; *Machine Learning* tools; ecological data; prediction.

Sommaire

Introduction générale



CHAPITRE 1

1. Le changement climatique global

Le fait que nous assistions actuellement à un changement global du climat de la planète est indiscutable. Il existe au sein de la communauté scientifique un vaste consensus à propos du fait que le système climatique terrestre - formé par l'atmosphère, les océans, la biosphère, la lithosphère et la cryosphère - vit des changements qui ne sont pas attribuables uniquement à la variabilité interne du système (IPCC, 2007). Les pressions externes à caractère naturel (la variabilité de l'activité solaire, les éruptions volcaniques, etc.) sont insuffisantes pour expliquer de manière satisfaisante les altérations observées, on ne peut comprendre ces dernières que si l'on tient compte des pressions de type anthropiques (Karl and Trenberth, 2003).

La température de la surface terrestre a augmenté de près de 0,8ºC depuis 1850. Les émissions de gaz à effet de serre, et en particulier celles de CO_2, sont les principales responsables de cette augmentation de la température. L'augmentation du contenu calorifique des océans, l'augmentation du niveau de la mer de 1 à 2 mm/an, ainsi que le recul des glaciers continentaux et de la couche de glace de l'Arctique sont d'autres effets observés très probablement associés aux pressions anthropogéniques (Bindoff et al., 2007). À ces changements, il faut ajouter l'augmentation du risque d'inondations côtières, une plus grande variabilité de la fréquence des pluies (avec des épisodes de fortes précipitations et des périodes de sécheresse plus longues), une augmentation de l'intensité des ouragans, et des changements dans la distribution de nombreuses espèces dans des zones côtières (IPCC, 2007).

Bien que les espèces aient fait face à des variations du climat tout au long de leur histoire évolutive, un point clé des changements actuels est la rapidité avec laquelle ils se produisent. Les changements régionaux sont souvent plus importants dans le contexte des réponses écologiques au changement climatique que les moyennes globales (Walther et al., 2002). L'étude des impacts du changement climatique ne doit donc pas se focaliser uniquement sur des échelles globales mais aussi sur des échelles régionales et locales comme par exemple les zones côtières, les bassins des fleuves et des rivières ou les estuaires. (IPCC, 2007).

2. Les effets du changement climatique sur les écosystèmes marins

Les océans accumulent l'énergie de la radiation solaire et l'échangent avec l'atmosphère. Étant donné qu'ils couvrent plus du 70% de la surface de la Terre, les océans et les mers continentales jouent un rôle clé comme régulateurs du climat car ils représentent le plus grand réservoir et distributeur de chaleur (IPCC, 2007). Ils forment en outre la première source d'eau du globe, ce sont les principaux régulateurs de l'évaporation, des précipitations et de la vapeur d'eau dans l'atmosphère et ils contribuent à la formation, à la distribution et à la fonte des glaces marines (IPCC, 2007). Finalement, ils constituent le premier réservoir des gaz à effet de serre, comme le dioxyde de carbone (CO_2), par le biais d'un système biologique actif qui joue un rôle crucial dans le cycle du carbone (IPCC, 2007).

La forte densité et capacité calorifique des océans, en comparaison de l'atmosphère, en font un élément d'amortissement des changements atmosphériques, ce qui est illustré par exemple, par le fait que ils ont absorbé 84% de la chaleur retenue par la Terre dans le processus de réchauffement global (Levitus *et al.,* 2005). Ceci a induit l'augmentation de leur température de surface, qui s'est élevée de 0,13^{o}C par décennie depuis 1979, et on prévoit que cette tendance continuera ou s'accentuera même dans le futur (IPCC, 2007). En outre, le climat de la planète dépend de sa capacité à redistribuer l'excédent d'énergie absorbée dans des latitudes basses vers des latitudes hautes où le bilan énergétique est négatif. Ce transport de chaleur se fait aussi bien par le biais de l'atmosphère que des océans, ces derniers utilisent un système de courants à l'échelle planétaire que l'on connaît sous le nom de circulation thermohaline (Rahmstorf, 2003).

L'impact des pressions d'origine anthropique a été documenté dans les écosystèmes marins de l'ensemble du globe (Halpern et al., 2008, Fig. 1) y compris les estuaires (Lotze et al., 2006), les écosystèmes pélagiques (Worm et al., 2006), les récifs coralliens (Hughes et al., 2003), les écosystèmes côtiers (Jackson et al., 2001 ; Harley et al., 2006), les plateformes continentales (Auster, 1998) et les écosystèmes abyssaux (Koslow, 2007).

Les causes sont variées parmi lesquelles on peut mentionner l'introduction d'espèces, la pollution, la surpêche et la destruction d'habitats, l'eutrophisation et l'acidification des océans et le changement climatique dont on a fait état précédemment (IPCC, 2007).

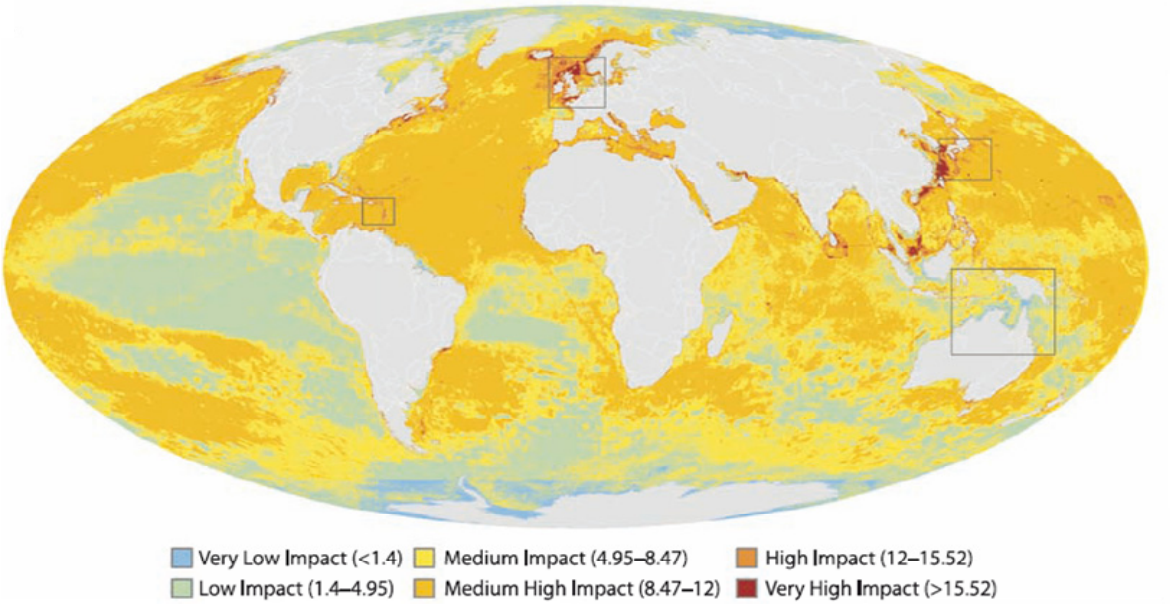


Figure 1. Carte globale qui illustre l'impact humain sur les écosystèmes marins. Les carrés indiquent des zones à impact très élevé et très faible. D'après Halpern et al. (2008).

Les réponses écologiques à ces perturbations sont aussi très variées et peuvent comprendre des modifications de la distribution (depuis des modifications de la zonation jusqu'à des modifications des rangs biogéographiques) et de la composition des espèces, des modifications de la diversité et de la structure des communautés, des modifications des productions primaire et secondaire ainsi que de la dynamique et l'évolution des populations (Harley et al., 2006).

Au sein des écosystèmes marins, les zones côtières semblent être gravement touchées, du fait d'une concentration des activités et impacts d'origine anthropique (37% de la population mondiale vit à moins de 100 km des côtes, Gattuso et al., 1998), mais également par une sensibilité accrue aux évènements climatiques extrêmes et de manière générale, à l'augmentation de la variabilité du climat (Schar et al., 2004).

3. La Méditerranée

Le bassin de la Méditerranée est le plus grand (2 969 000 km^2) et le plus profond (moyenne 1 460 m, maximum 5 267 m) de la planète (Coll et al., 2010). Entourées par l'Afrique, l'Europe et l'Asie, les côtes de la Méditerranée ont été témoin de la prospérité et du déclin de plusieurs civilisations. La région a été une route importante pour les commerçants et les voyageurs des temps anciens, elle a permis des échanges commerciaux et culturels. Ses côtes accueillent un grand nombre d'habitants distribués dans 21 pays et elles sont

devenues l'une des principales destinations touristiques du monde, elles reçoivent environ 200 millions de touristes par an (NOAA, 2009).

La Méditerranée communique avec l'océan Atlantique à travers le Détroit de Gibraltar par l'ouest et avec la mer de Marmara et la Mer Noire à travers le Détroit des Dardanelles au nord-est. Au sud-est, le Canal de Suez permet la communication de la Méditerranée avec la Mer Rouge et l'océan Indien. Le Détroit de Sicile sépare l'île de Sicile de la côte de la Tunisie et divise la mer en deux sous-régions principales : la région occidentale (surface = 0,85 millions km2) et la région orientale (surface = 1,65 millions km2) (Coll et al., 2010). En ce qui concerne les conditions océanographiques de base, la Méditerranée est un bassin de concentration car l'évaporation dépasse les précipitations et les apports par les fleuves et ruissellements (Bethoux, 1979 ; Hopkins, 1985). L'évaporation est plus forte dans la région est, elle provoque une diminution du niveau de la mer et une augmentation de la salinité d'ouest en est (Coll et al., 2010). Le gradient de pression qui en résulte pousse des eaux relativement froides et à la salinité relativement faible depuis l'Atlantique vers la Méditerranée. Cette masse d'eau se chauffe à mesure qu'elle avance vers l'est où elle devient plus salée, ce qui provoque son enfoncement, avant son retour vers l'ouest pour sortir finalement à travers le Détroit de Gibraltar (Hopkins, 1985). Le climat de la région se caractérise par des étés chauds et secs et des hivers froids et humides. La température moyenne annuelle de la surface de la mer montre une saisonnalité marquée et des gradients clairs d'ouest en est et du nord au sud (Margalef, 1985 ; Coll et al., 2010, Fig. 2a). Le bassin est en général oligotrophes bien que l'on observe des gradients, la production biologique décroît du nord vers le sud et de l'ouest vers l'est et s'avère inversement proportionnelle à la température et à la salinité (Margalef, 1985 ; Coll et al., 2010, Fig. 2b).

Les eaux côtières représentent 20% du total des eaux méditerranéennes, ce pourcentage est trois fois plus élevé que celui qui correspond à la totalité des océans. Les eaux côtières de la Méditerranée jouent donc un rôle proportionnellement plus important (Pinardi et al., 2006).

La mer Méditerranée possède une riche histoire géologique qui comprend un isolement des océans, ce qui a failli provoquer son assèchement pendant la crise messinienne (il y a 5,96 millions d'années) (Maldonado, 1985 ; García-Castellanos et al., 2009). Son histoire géologique, biogéographique et écologique ainsi que son histoire humaine ont contribué à l'importante richesse biologique et culturelle de la mer Méditerranée (Fredj et al., 1992 ; Myers et al., 2000 ; Boudouresque, 2004 ; Danovaro et Pusceddu, 2007).

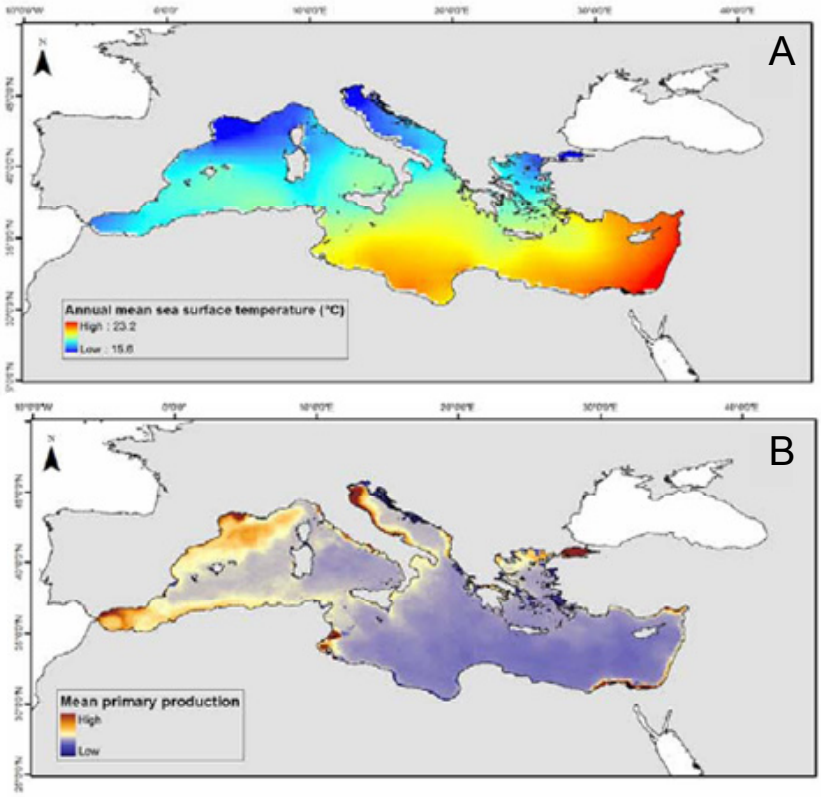


Figure 2. Caractéristiques océanographiques de la Méditerranée. (A) Température moyenne annuelle des eaux superficielles (^{o}C) (2003, NOAA), (B) Moyenne annuelle de la production primaire relative. D'après Coll et al. (2010).

4. Les principales caractéristiques hydrologiques de la Méditerranée nord-occidentale (MNO)

Les principales caractéristiques hydrodynamiques de la Méditerranée nord-occidentale (MNO) ont été sujettes à plusieurs études et ont déjà été décrites (ex. Hopkins, 1985; Millot, 1990).

Les forces dominantes dans cette région sont: 1) la présence de forts vents orographiques provenant du nord-ouest (Mistral) et du nord (Tramontane), ce qui provoque des remontées des eaux froides et profondes ainsi que des enfoncements et des oscillations inertielles (Millot, 1979, 1990; Petrenko, 2003); 2) le Courant du Nord qui se déplace le long de la limite de la plateforme continentale mais qui peut aussi y pénétrer et provoquer des modèles complexes de circulation dans la zone côtière et 3) l'entrée d'eau douce du Rhône (flux moyen de 1 700 m^3 s^{-1}), dont le panache peut s'étaler jusqu'au Cap de Creus (Espagne) vers l'ouest (Salat et Pascual, 2002) et jusqu'au Golfe de Marseille vers l'est (Younes et al.,

2003), ainsi que d'autres rivières ou de fleuves locaux qui peut s'avérer considérable lors des saisons de pluie (Bensoussan et al., 2010).

Les eaux de la MNO se caractérisent par une saisonnalité marquée. En hiver, (de décembre à mars) la température de l'eau diminue et atteint un minimum de près de 13°C en mars. Après cette période froide, la température augmente lentement jusqu'à la formation de la thermocline (Margalef, 1985). Ainsi, dans les eaux superficielles de la MNO, la température varie entre 13°C en hiver et 26°C en été (Margalef, 1985) bien que l'on remarque une variabilité spatiale (Bensoussan et al., 2010). Les eaux profondes présentent une température proche de 13°C qui se maintient relativement constante (Margalef, 1985).

Box 1 – Les conditions de température des régions étudiées dans la thèse

Séries de température disponibles dans les eaux côtières de la MNO

Il existe peu de séries de température pour les zones côtières de la MNO. En général, elles ne reprennent que les 30 ou 40 dernières années. L'exemple le plus significatif est la série des Îles Medes qui a compilé, sans interruptions, depuis le début des années 70 et avec une fréquence presque hebdomadaire, des données de température entre 0 et 80 mètres de profondeur grâce à l'initiative de M. Josep Pascual (voir www.meteoestartit.cat pour détails). Sur la côte française méditerranéenne, nous disposons des séries de trois municipalités liées au *Service d'observation en milieu littoral* (SOMLIT http://somlit.epoc.u-bordeaux1.fr) (Villefranche-sur mer, Marseille et Banyuls) et de la série de l'île du Levant obtenue par la Marine Nationale française. Pour connaître les conditions thermiques antérieures aux années 1970, nous ne disposons que de la série de surface du marégraphe de Marseille qui englobe la période allant de 1884 à 1967 (Romano et Lugrezi, 2007; Bensoussan et al., 2009; Romano et al., 2010). Malgré l'importance des données réunies dans les séries mentionnées, il existe un vide relatif à la connaissance du régime thermique des eaux côtières de la MNO en raison du manque de données couvrant une échelle spatiale et temporelle adéquate. Étant donné que la température des eaux côtières est très variable d'un point de vue spatial et temporel, surtout pendant l'été (Bensoussan et al., 2010), en partie en raison des conditions hydrodynamiques, météorologiques et orographiques (Margalef, 1985) mais aussi en raison de facteurs locaux, comme l'orientation de la côte ou le profil bathymétrique (Bensoussan et al., 2010), il s'avère nécessaire de disposer de séries à haute résolution temporelle (horaire) et spatiale.

Pour répondre à ce besoin, depuis le début de 1999, le Centre d'Océanologie de Marseille a lancé une initiative en vue de l'acquisition de séries de température à haute résolution (horaire) dans les eaux côtières (0 – 40m) de la MNO, en incluant des zones allant du nord-ouest de la Corse aux côtes de la France, de l'Espagne et des îles Baléares. Les premiers registres disponibles ont été obtenus par le Dr. J. G. Harmelin (UMR 6540 DIMAR, Centre d'Océanologie de Marseille) dans deux localités de la côte provençale française. Ils existent actuellement 18 localités équipées de thermomètres qui couvrent différentes zones de la MNO et profondeurs entre 5 et 40 mètres (tout les 5 mètres).

A l'initiative du groupe MedRecover de l'Insitut de Ciències del Mar-CSIC (Espagne), le réseau t-MedNet a récemment été développé pour promouvoir l'acquisition et l'analyse de séries de température de haute résolution (http://www.t-mednet.org).

Les régimes thermiques des régions étudiées dans cette thèse

Les régions étudiées au cours de cette thèse ont été sélectionnées car elles disposaient de séries de température au cours de tous les épisodes de mortalité massive, ou de certains d'entre eux (1999, 2003 et 2006, section 6.2). Il s'agit, d'ouest en est : du Parc Natural del Montgrí, Illes Medes i Baix Ter (L'Estartit, Espagne); Riou (Marseille, France); du Parc National de Port-Cros (France) et de la Réserve naturelle de Scandola (Corse, France) (Fig 3b). Pendant l'été, les conditions les plus chaudes sont observées dans les 15 premiers mètres de profondeur sur la côte ouest de la Corse (Scandola), cet endroit montrant une stratification permanente de la colonne d'eau et des oscillations de la thermocline entre 15 et 30 m (Bensoussan et al., 2010). Au contraire, la région de Marseille (Riou), qui est située près du canyon de Cassidaigne (la plus intense des sept sources de upwellings de la Méditerranée nord-occidentale, Millot, 1979), montre des étés plus froids, avec une grande variabilité dans le régime thermique ainsi qu'une forte variabilité interannuelle due à des affleurements récurrents d'eaux profondes et froides ($T<16^{o}C$) qui dépendent de l'action du vent (Bensoussan et al., 2010). Lorsque l'on compare Riou (Marseille) et Port-Cros (Alpes-Maritimes), qui se trouve 80 km plus à l'est, on remarque des différences claires dans les régimes thermiques estivaux, ce qui prouve une forte variabilité spatiale. Port-Cros subit l'influence des eaux chaudes conduites vers l'ouest par le Courant du Nord (Sparnocchia, 2006) et par des masses d'eaux froides issues des remontées provoquées par le Mistral.

Ceci fait que la structure thermique de Port-Cros se trouve dans une position intermédiaire en ce qui concerne le régime thermique estival, entre Scandola et Riou, elle peut varier au cours de l'année selon les périodes plus ou moins longues où souffle le Mistral (Bensoussan et al., 2010). Dans les Îles Medes (côte catalane, Espagne), la caractéristique principale est l'approfondissement de la thermocline estivale par pulses, la profondeur de mélange de 40 m est assez fréquente (Salat et Pascual 2002; Bensoussan et al., 2010).

Afin d'illustrer la variété des caractéristiques thermiques des différentes régions, la Figure 3c présente les profils de température de l'été 2005 entre 5 et 40 m de profondeur pour chacune des quatre régions (voir Bensoussan et al. (2010) pour détails).

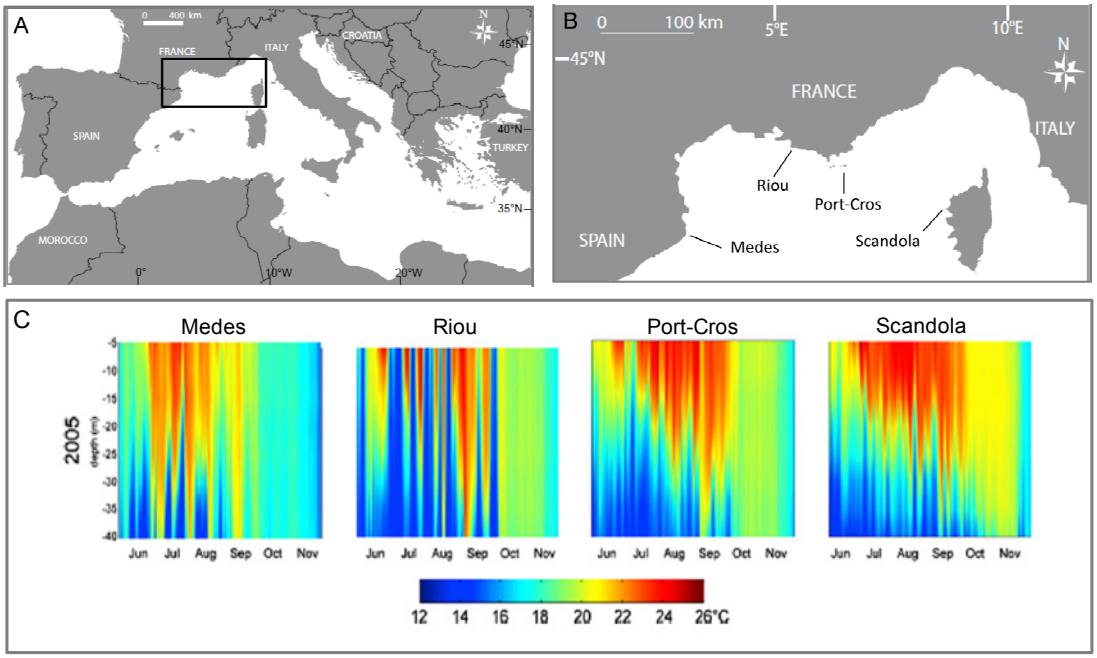


Figure 3. Mer Méditerranée et limites de la zone d'intérêt de la présente thèse en Méditerranée nord-occidentale (A), présentation des quatre sites d'étude (B), séries temporelles de la température moyenne journalière (5-40 m) de chaque site d'étude de juin à novembre de 2005 (d'après Bensoussan et al., 2010) (C).

5. Le changement climatique dans la MNO

Dans le sud de l'Europe, les projections de l'IPCC (2007) indiquent une augmentation de la température et de la fréquence des sécheresses dans une région qui est déjà vulnérable à la variabilité du climat. L'une des manifestations du changement climatique en Europe est la présence de vagues de chaleur (Beniston, 2004; Schar et al., 2004; Rebetez et al., 2009) qui se caractérisent autant par l'augmentation des températures moyennes que par leur

variabilité (Schär et al., 2004). L'une des caractéristiques de ces événements consiste en de longues périodes d'absence de vents forts qui provoquent des anomalies thermiques dans les eaux côtières (Romano et al., 2000; Sparnocchia et al., 2006). Selon les projections actuelles, la température de la surface de la MNO devrait augmenter en moyenne de 2 à 4°C à la fin de ce siècle (Somot et al., 2008).

À partir des séries de température disponibles (voir Box 1), nous avons observé une augmentation de la température des eaux superficielles d'entre +0,8°C (station de Villefranche-sur-mer, Prieur, 2002; séries Naval de l'Île du Levant, France) et +1,02°C (station de L'Estartit, Salat et Pascual, 2002) au cours de 28 années (1974 – 2001) (Fig. 4). Ces taux de réchauffement sont cohérents par rapport aux prédictions d'augmentation moyenne pour la fin du XXIe siècle (voir plus haut Somot et al. 2008). Il est également intéressant de signaler que l'analyse de la série du marégraphe de Marseille (de 1884 à 1967; Romano and Lugrezi, 2007; Romano et al., 2010) montre une tendance à l'augmentation de 0,7 à 0,8°C en 100 ans, soit un taux de réchauffement environ trois fois inférieur à celui observé au cours de ces 30 dernières années. Ceci pourrait indiquer une accélération du réchauffement pour la MNO.

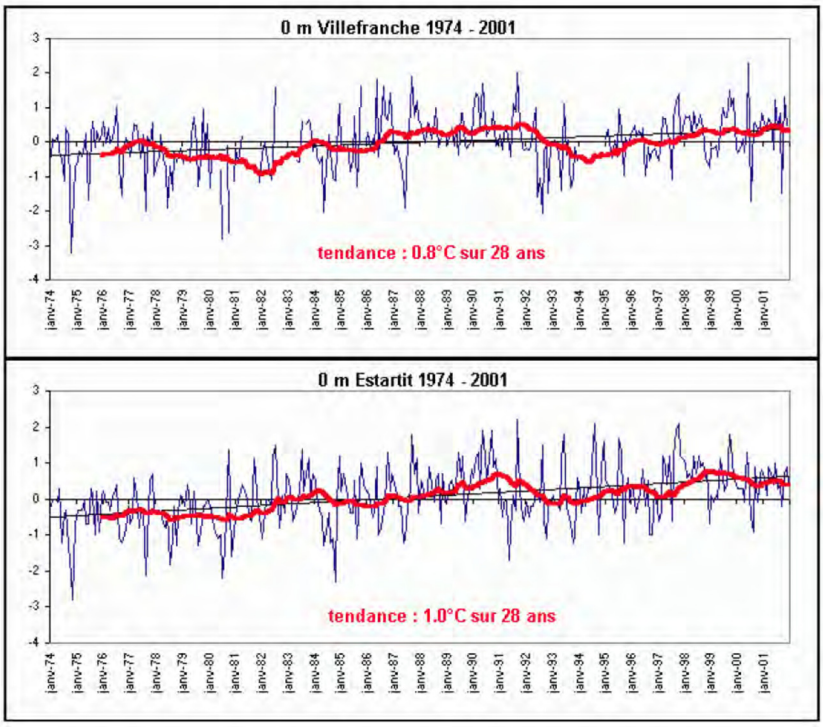


Figure 4. Écarts mensuels à la moyenne pluriannuelle (bleu) avec moyenne mobile (rouge) et estimation de la tendance linéaire depuis 1974 à 2001 de las séries de température de Villefranche-sur-Mer (Prieur, 2002) et de la station de L'Estartit (Salat et Pascual, 2002). D'après de Bensoussan and Romano, in Boury-Esnault et al., 2006.

Les eaux profondes de la Méditerranée ont également montré un réchauffement depuis 1959 (+ 0,12°C, Bethoux et al. 1990). Des études récentes d'intégration de séries de température sur les côtes espagnoles depuis 1900 indiquent une tendance significative en ce qui concerne l'augmentation de la température aussi bien des couches superficielles (0 – 200 m), intermédiaires (200 – 600 m) que profondes (>600 m) (Vargas-Yáñez et al., 2010).

Outre les tendances de réchauffement, on prévoit aussi une prolongation des conditions de stratification dans les eaux côtières. En effet, des analyses récentes de séries de température de 30 ans (Salat et Pascual, 2002) ont indiqué une prolongation de 40% des conditions de stratification entre 1974 à 2006 (Coma et al., 2009).

6. Les effets du changement climatique dans la MNO

6.1. La diversité des espèces

Bien qu'elle n'occupe que 0,82% de la superficie des océans et 0,32% de leurs volumes, la mer Méditerranée est considérée comme un point chaud (*hotspot*) de la biodiversité (Bianchi et Morri, 2000; Coll et al., 2010). On a recensé environ 17 000 espèces marines bien que les estimations de diversité soient très probablement encore incomplètes (Coll et al., 2010). La plupart des espèces décrites se concentrent dans des environnements côtiers benthiques tels que les fonds rocheux et les herbiers de *Posidonia* (Boudouresque, 2004).

On considère actuellement que la destruction de l'habitat ou sa dégradation, la surpêche, la pollution, le changement climatique, l'eutrophisation et l'introduction d'espèces invasives sont les facteurs majeurs qui touchent un grand nombre de groupes taxonomiques (Coll et al., 2010). Ces perturbations sont appelés à se renforcer, et notamment celles associées à la dégradation de l'habitat et au changement climatique (Coll et al., 2010). Ce dernier peut en outre agir en synergie avec certains des autres facteurs précédemment mentionnés, accentuant ainsi leurs effets. Par exemple, le réchauffement observé dans le bassin méditerranéen a augmenté le nombre d'espèces invasives capables de s'établir et a permis à des espèces provenant d'eaux plus chaudes (autochtones ou exotiques) qui se trouvent déjà dans la Méditerranée d'accroître leurs aires de distributions (Chevalodonne et Lejeusne, 2003; Coll et al., 2010). L'hypothèse d'une tropicalisation de la mer Méditerranée a ainsi été émise en raison de la présence de plus en plus importante d'espèces invasives d'origine tropicale (Bianchi et Morri, 2003).

6.2. Les événements de mortalité massive et le changement climatique

Au cours des étés de 1999 et 2003, deux événements de mortalité massive (EMM) sans précédents ont touché les communautés benthiques de la Méditerranée nord-occidentale (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009). Des EMM avaient déjà été répertoriés en Méditerranée (Harmelin, 1984; Bavestrello et Boero, 1986; Coma et al., 2009). Cependant la grande échelle spatiale (des milliers de km de côte dans le bassin nord-occidental allant des îles Baléares à la mer de Ligurie, en passant par la Catalogne, le Golfe de Lion, la Corse et la Sardaigne, Fig. 5) (Cerrano et al., 2000; Pérez *et al.* 2000; Garrabou et al., 2009) et le grand nombre d'espèces touchées (environ trente) différencient les événements de 1999 et 2003 de ceux observés précédemment. Les EMM se sont produits parallèlement à des anomalies thermiques positives dans la colonne d'eau. En 1999, le régime thermique s'est caractérisé par un réchauffement de 2 à 3°C, qui a atteint une profondeur de 30 – 40 m et a été associé à une grande stabilité (Romano et al., 2000). En 2003, les eaux superficielles ont été entre 1 et 3°C plus chaudes que les moyennes des années précédentes, atteignant la température de 28°C dans les régions les plus chaudes (Garrabou et al., 2009).

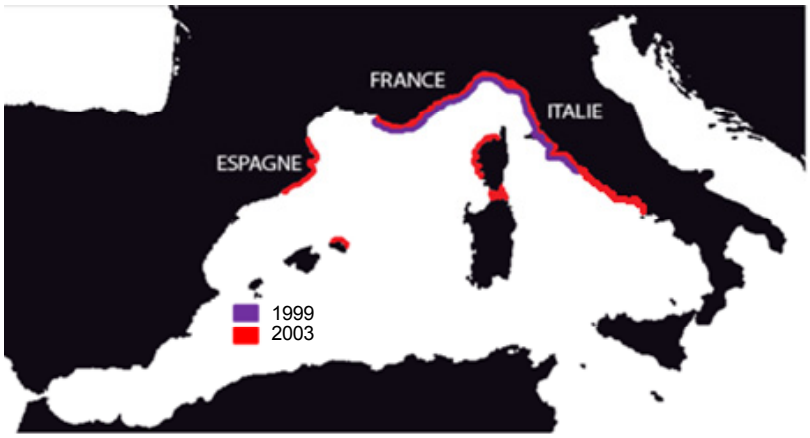


Figure 5. Aires impactées par les événements de mortalité massive observés en 1999 et 2003.

Parmi les organismes les plus touchés, on soulignera différentes espèces d'éponges et de gorgonaires (Fig. 6). Certaines de ces espèces sont considérées comme des espèces clé de l'une des communautés les plus emblématiques de la Méditerranée: le coralligène. La communauté du coralligène est connue pour sa richesse spécifique (environ 1 600 espèces ont été identifiées, 10% de toutes les espèces recensées en Méditerranée) et sa grande complexité structurelle (Ballestros 2006). Dans le cas des gorgonaires, les épisodes de mortalité se traduisent par une nécrose totale ou partielle des colonies (Fig. 6).

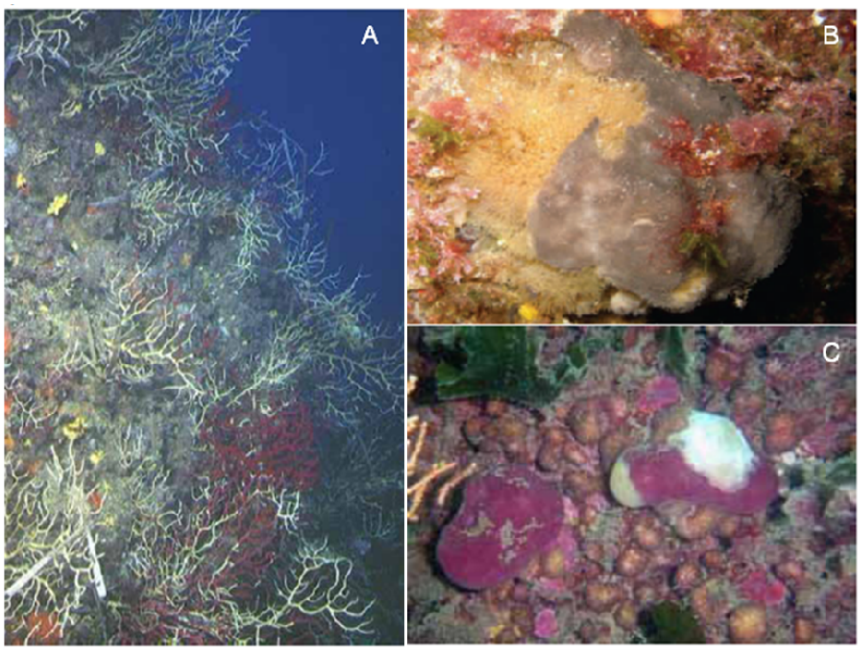


Figure 6. Espèces de macro-invertébrés benthiques parmi les plus affectés par les événements de mortalité massive: la gorgone *Paramuricea clavata* (les axes blancs correspondent à des colonies mortes) (A), le spongiaire *Spongia officinalis* (la nécrose correspond aux parties marrons claires) (B), le spongiaire *Petrosia ficiformis* (la nécrose correspond à la partie blanche) (C). D'après de Garrabou et al., 2009 et de Ledoux, 2010.

Lors des épisodes de mortalité, on a observé une grande variabilité du niveau d'impact aussi bien entre colonies d'une même population (ex. des colonies tout à fait mortes aux côtés de colonies intactes), qu'entre populations, et ce aussi bien au niveau intrarégional qu'interrégional. Par exemple, les populations de corail rouge *Corallium rubrum* de la région de Marseille montrent des niveaux d'affectation qui varient entre 5 et 80% (Garrabou et al., 2001). On a pu observer des différences du même ordre de grandeur lorsque l'on compare la moyenne du pourcentage de colonies touchées de différentes populations de la gorgone rouge *Paramuricea clavata* entre différentes régions de la MNO (Garrabou et al., 2009). Si l'on tient compte du fait que la plupart des espèces touchées, et en particulier les gorgonaires, sont des espèces longévives, avec des dynamiques de populations lentes (ex. Linares et al., 2010a, b), leur capacité de récupération est *a priori* modérée. L'augmentation probable de la fréquence des EMM au cours des prochaines décennies invite donc à s'interroger quant à la persistance de ces communautés dans les zones les plus touchées par le réchauffement (0-50 m de profondeur) (Garrabou et al., 2009).

On a avancé différents mécanismes qui incluent la température de manière directe ou indirecte comme principaux responsables de la mortalité observée au cours des EMM, surtout en ce qui concerne les gorgonaires.

Les faibles concentrations d'alimentation, les caractéristiques de la période estivale, en combinaison avec la présence de températures anormales pourraient aussi produire un stress physiologique qui déboucherait sur la mort de ces organismes (Coma et al., 2009).

L'apparition de microorganismes pathogènes thermodépendants offre une autre explication quant au développement de la mortalité (Bally et Garrabou, 2007 ; Vezzulli et al., 2010). Finalement, l'apparition de températures anormalement élevées (≥26°C) pourrait provoquer des dysfonctions métaboliques et la mort des organismes (Torrents et al., 2008).

Les mécanismes mentionnés n'en excluent pas d'autres car ils pourraient agir en synergie et produire une accélération du processus de mortalité.

Box 2 – *Paramuricea clavata* comme espèce modèle

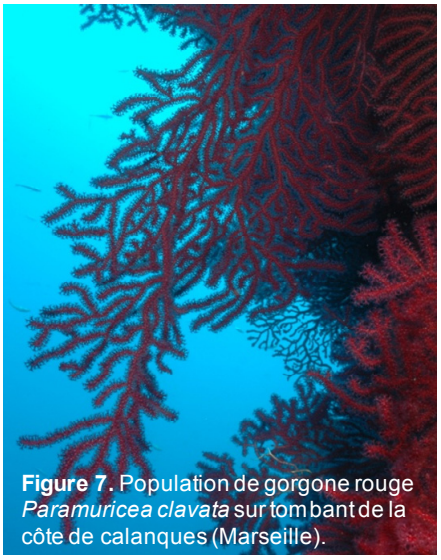


Figure 7. Population de gorgone rouge *Paramuricea clavata* sur tombant de la côte de calanques (Marseille).

La gorgone rouge *Paramuricea clavata* (Risso, 1826) (Cnidaria, Anthozoa, Octocorallia) (Fig. 7) a été choisie comme modèle biologique principal pour répondre à certaines des questions de cette thèse (voir section 9). *P. clavata* est une espèce endémique de la Méditerranée qui forme l'un des paysages les plus emblématiques des communautés de coralligène. Le coralligène qui, comme on l'a rappelé plus haut, se caractérise par la richesse de ses espèces et sa forte complexité structurelle (Ballestros, 2006) est un substrat d'origine biogénique produit surtout par l'accumulation d'algues calcaires incrustantes, qui grandissent dans des conditions de faible intensité de lumière. Bien qu'elle soit plus étendue dans la zone circalittorale, elle peut aussi se développer dans la zone infralittorale lorsque la lumière est suffisamment faible pour permettre la croissance d'algues calcaires. Les formations infralittorales du coralligène se développent sur des parois verticales, dans des canaux profonds et saillants et occupent de petites surfaces (Ballestros, 2006).

Les suspensivores passifs font partie du coralligène et on soulignera parmi eux, de par leur abondance, les populations de différents gorgonaires (Zabala et Ballestros, 1989). Les populations de gorgone rouge sont celles qui contribuent le plus en termes de biomasse ainsi que de structuration des communautés où elles se développent (Gili et Coma, 1998).

P. clavata a été l'une des espèces les plus touchées par différentes perturbations liées à l'activité humaine (Harmelin et Marinopoulus, 1994; Mistri et Cecherelli, 1996; Bavestrello et al., 1997; Coma et al., 2004; Guliani et al., 2005) mais aussi par les EMM observés récemment dans la MNO (Cerrano et al., 1999; Pérez et al., 2000; Garrabou et al., 2009). On peut donc considérer à *P. clavata* comme un bon indicateur de la magnitude et de l'intensité de perturbations qui touchent toute la communauté (Linares, 2006).

P. clavata se trouve dans des habitats dont l'intensité et la luminosité sont restreintes (valeurs d'irradiance entre 0,12 et 27,6%), on la retrouve surtout sur des parois verticales ou des surfaces horizontales exposées à de forts courants dans la zone circalittorale (15-80 m) (Weinberg, 1975, 1980; Zabala et Ballestros, 1989; Ballestros, 2006).

P. clavata est une espèce longévive (elle peut vivre > 50 ans) et qui présente une dynamique de populations lente avec des taux de croissance d'environ 0,8 cm par an (Coma et al., 1998). La croissance est saisonnière et atteint son maximum au printemps, ce qui correspond à la fluctuation saisonnière de la disponibilité d'aliment (Coma et al., 1998a). Les taux de mortalité de la gorgone rouge sont plus faibles (0-2%) lorsque les populations ne sont pas exposées à des perturbations anthropogéniques. La mortalité totale d'une colonie peut être due à des détachements du substrat ou à des lésions. Les principales causes de détachement provoquées par l'homme comprennent les ancrages, l'appareillage de pêche et la plongée (Harmelin et Marinopoulus, 1994; Bavestrello et al., 1997; Coma et al., 2004).

P. clavata est une espèce dioïque. La fécondation des oeufs est externe et se produit pendant les épisodes discrets de ponte entre les mois de juin et juillet (Coma et al., 1995; Linares et al., 2008). Les colonies sont sexuellement mâtures à partir de 10 cm de hauteur, ce qui correspond à un âge d'environ 7 à 13 ans en moyenne (Coma et al., 1995).

P. clavata est un suspensivore passif (ex. elle s'alimente de particules que les courants transportent), son régime alimentaire est hétérogène et incluant des nanoeucaryotes (3,8 µm) jusqu'à des copépodes (700 µm) en passant par différents ciliés, dinoflagellés, diatomées et zooplancton (nauplii, oeufs de copépodes et d'autres invertébrés, copépodes calanoïdes) ainsi que de la matière organique particulaire (Coma et al., 1994 ; Ribes et al., 1999). Les maxima dans la capture de proies ont été enregistrés au printemps et à la fin de l'automne (Coma et al., 1994). L'ingestion de carbone d'origine particulaire montre une saisonnalité marquée où l'hiver et le printemps sont les saisons aux taux d'ingestion les plus élevés (Ribes et al 1999). Les faibles taux de synthèse de nouveaux tissus pendant l'été, la contraction de polypes et les faibles niveaux de Q10 expliquent les faibles taux de respiration de *P. clavata* observés pendant cette période. Ces observations étayent l'hypothèse que les restrictions métaboliques doivent être associées à la léthargie pour l'activité de cette espèce pendant l'été (Coma et al., 2000; Coma et Ribes 2003; Coma et al., 2009).

À une échelle régionale, les menaces les plus importantes pour les populations de *P. clavata* sont la dégradation des habitats, la sédimentation, les *blooms* d'algues filamenteuses auxquels s'ajoutent les EMM enregistrés au cours de ces dernières années (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009). Des études de surveillance de populations touchées ont montré que les effets retardés de ces épisodes peuvent s'avérer encore plus graves que les effets immédiats (Linares et al., 2005). Des analyses des effets des EMM sur des populations de *P. clavata* à partir de modèles démographiques ont montré de faibles taux de croissance de la population et une probabilité d'extinction dans un laps de temps d'à peine quelques décennies (Linares et Doak, 2010).

7. Que savons-nous de la relation température - mortalité au sein des communautés benthiques de la MNO?

Bien que quelques écosystèmes aient été étudiés quant à la réponse des organismes au stress thermique, comme par exemple les récifs de corail (ex. Jokiel and Coles, 1990; Goreau et al., 1997; Fitt et al., 2001; Coles and Brown, 2003; Berkelmans et al., 2004; Jokiel, 2004; McClanahan et al., 2007), on sait très peu de choses sur l'effet de la température dans les communautés benthiques de mers tempérées et, en particulier, celles de Méditerranée. Les organismes sessiles ne peuvent «échapper» au stress thermique (défini comme une rupture dans les réactions enzymatiques provoquant des dysfonctions

biochimiques et métaboliques, Cossings et Bowler, 1987), ce qui laisse supposer des conséquences importantes pour le futur des communautés qui les abritent.

7.1. La thermotolérance des espèces

Parmi les communautés benthiques de la Méditerranée les plus touchées par les EMM, on soulignera les communautés du coralligène. L'observation des épisodes de mortalité associés à des anomalies thermiques positives (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009; Bensoussan et al., 2010) a conduit à étudier le rôle de la température dans ces processus. Au cours de ces dernières années on a réalisé plusieurs travaux expérimentaux surtout avec des espèces d'anthozoaires (Torrents et al., 2008; Coma et al., 2009; Previati et al., 2009; Ferrier-Pages, 2009; Rodolfo-Metalpa et al., 2006a, 2006b, 2010; Cebrian et al., 2011).

Les résultats de ces travaux indiquent que 25^{o}C est une température critique pour plusieurs espèces étudiées car des expositions relativement courtes à cette température peuvent provoquer une chute du taux de calcification, de l'activité des polypes, des taux de respiration et, dans de nombreux cas, produire la nécrose du tissu (Torrents et al., 2008; Previati et al., 2009). Cependant, malgré les généralisations que l'on peut réaliser à propos d'une possible température critique, *«il est clair que la limite supérieure de température pour la vie ne peut être définie de manière précise»*. Cette phrase de Schmidt et Nielsen (1996) donne une idée de la difficulté de proposer une valeur unique de thermotolérance pour différentes espèces ou populations. La température à elle seule ne peut pas déterminer les limites de la thermotolérance car une température peut être mortelle ou non selon le temps d'exposition (Cossins and Bowler, 1987; Jokiel and Coles, 1990, Fitt et al., 2001).

En fait, même s'il s'avère que 25^{o}C est une température critique pour plusieurs espèces macrobenthiques de la MNO, 24^{o}C s'est avéré nuisible pour certaines de ces espèces lors d'expositions expérimentales plus prolongées (Rodolfo-Metalpa et al., 2006a; Torrents et al., 2008).

En outre, comme on l'a indiqué dans la section 6.2, la température peut avoir une influence indirecte sur la mortalité en favorisant l'apparition d'organismes pathogènes thermodépendants (Bally et Garrabou, 2007). Cela peut aussi être le cas suite à une synergie avec d'autres facteurs comme la pénurie d'aliment qui provoque la mortalité par stress physiologique (Coma et al., 2009).

7.2. Variabilité dans la réponse à la température

Pendant les EMM observés dans le bassin méditerranéen, la réponse aux conditions anormales de température a beaucoup varié selon les individus, les populations et les régions (voir section 6.2). Ce type de variabilité dans la réponse aux anomalies thermiques a également été observée chez des coraux tropicaux pour lesquels on a remarqué des différences d'affectation entre espèces (Jokiel et Coles, 1990) et entre populations d'une même espèce (Jokiel et Coles, 1990). Ceci prouve la complexité de l'interprétation de la relation température – affectation.

Parmi les explications possibles à la variabilité dans la réponse à la température, il y a des processus d'adaptation locale ou d'acclimatation.

L'adaptation locale fait référence aux patrons et processus résultant de l'action de la sélection divergente qui induit la formation de génotypes localement adaptés, c'est à dire ayant une plus grand valeur adaptative moyenne (*fitness)* dans leur habitat que les génotypes étrangers (Williams, 1966; Kawecki et Ebert, 2004). D'autre part, l'acclimatation est la capacité de modifier des traits du métabolisme comme réponse à des changements environnementaux (Wilson et Franklin, 2002). En plus de l'adaptation locale et de l'acclimatation, un nouveau concept est récemment apparu, celui d'évolution contemporaine qui fait référence à l'évolution de caractères héréditaires à l'intérieur des populations au cours de périodes de temps réduites d'une dizaine de générations (Hendry et Kinnison, 1999).

Des études récentes de la structure génétique des populations de deux gorgonaires de la MNO (*C. rubrum* et *P. clavata*) ont identifié une structuration génétique significative à différentes échelles spatiales, en incluant des échelles réduites de quelques dizaines de mètres seulement (Ledoux., et al., 2010; Mokhtar-Jamaï et al., 2011), ce qui serait compatible avec des processus d'adaptation locale. L'existence d'une adaptation locale chez ces organismes pourraient expliquer les réponses différentielles au stress thermique observées, par exemple, entre des populations provenant de profondeurs différentes (Torrents et al., 2008).

8. L'utilisation de modèles statistiques pour étudier la corrélation de données de température et de mortalité

La disponibilité de séries de température de haute fréquence (séries de température dont bénéficie cette thèse, voir Box 1) ainsi que la disponibilité de données de taux de mortalité enregistrés lors d'années avec et sans EMM obtenus par des chercheurs de différents centres de recherche (Centre d'Océanologie de Marseille, France; Institut de Ciències del Mar, Barcelone, Espagne; Departament d'Ecologia - Universitat de Barcelona et Centre d'Estudis Avançats de Blanes) ont permis pour la première fois d'étudier, à une échelle régionale, la relation statistique entre la température et la mortalité. L'objectif final de ces analyses est de quantifier la corrélation entre ces deux variables et de pouvoir développer des modèles prédictifs du risque d'épisodes de mortalité à partir des conditions de température.

À cette fin, on a choisi, dans le domaine de la statistique des techniques d'apprentissage *statistique* ou *Machine Learning* (ML). Les techniques de ML traitent les problèmes les plus classiques de la statistique: la classification, la régression, la décision, etc. Ce qui caractérise ce groupe de modèles ce ne sont pas leurs objectifs mais leurs stratégies qui utilisent massivement des algorithmes et des ressources informatiques pour travailler avec de grandes quantités de données dans de nombreux cas et/ou variables. En général, les techniques de ML ne supposent aucun type de distribution dans la variable dépendante. Cette restriction apparaît en général dans d'autres modèles (ex. des modèles linéaires), et ne correspondent souvent pas aux données collectées. En Écologie, les techniques ML se sont avérées très utiles dans l'étude de différents problèmes. Certaines des applications des techniques de *Machine Learning* incluent l'étude de la relation de la présence/absence d'espèces (ex. Ryder et Irwin, 1987 ; Cutler et al., 2007) et de l'abondance d'organismes en fonction de caractéristiques environnementales (biotiques ou abiotiques) (ex. Ryder et Irwin, 1987 ; Defeo et Gomez, 2005 ; Cutler et al., 2007). D'autres applications de ces modèles ont été la prédiction de *blooms* d'algues (Ribeiro et Torgo, 2008) et le recrutement de poissons (Fernandes et al., 2010). En résumé, les techniques de ML sont une bonne alternative pour quantifier le risque de EMM en fonction des conditions de température afin d'offrir un outil fiable de prédiction de ces épisodes (pour plus de détails sur la théorie liée à ces modèles et leur utilité pour l'analyse de données en écologie, voir le chapitre 4).

9. Problématique, objectifs et organisation de la thèse

L'objectif général de cette thèse est d'aller plus loin dans la connaissance du rôle de la température lors des EMM à l'échelle de la Méditerranée nord-occidentale, en mettant l'accent sur la compréhension de la variabilité d'impact observée dans différentes régions, et ce, afin de pouvoir anticiper les effets du changement climatique sur les communautés benthiques de substrat rocheux.

Le fait que les EMM observés au cours des dernières années dans la MNO se soient produits en même temps que des anomalies thermiques, ainsi que la tendance à l'augmentation de température détectée dans les eaux côtières de la MNO nous poussent à nous poser des questions à propos du futur des communautés benthiques touchées dans le contexte du réchauffement actuel.

L'une des caractéristiques des épisodes de mortalité est la forte variabilité inter-régionale observée, c'est pourquoi comprendre le rôle de la température dans les épisodes de mortalité implique de connaître les causes de cette variabilité.

Cette thèse aborde, en premier lieu, l'étude des anomalies thermiques dans différentes localités de la MNO pour détecter si les niveaux de stress thermique des différentes zones ont été ou non équivalents, afin de déterminer si ces possibles différences peuvent expliquer la variabilité dans l'impact observé.

Deuxièmement, à travers des expériences de thermotolérance on étudie les aspects de base de la thermotolérance de *P. clavata*, et la réponse à la température de populations de cette espèce provenant de régions et de profondeurs dont les régimes thermiques sont contrastés. On souhaite ainsi savoir si les populations de différentes régions et profondeurs sont adaptées au régime thermique des régions ou profondeurs où elles habitent et si elles montrent une réponse différentielle à la température susceptible d'expliquer les différences observées au cours des EMM.

Egalement et tenant compte de l'information qu'offrent les études préalables sur le stress physiologique associé à la période estivale chez les organismes benthiques sessiles, on a voulu étudier l'effet du stress physiologique dans la réponse à la température, en comparant la réponse de populations collectées à différents moments de l'année.

C'est la première fois que l'on réalise une expérience à ces échelles spatiale et temporelle pour la MNO.

Finalement, à partir de la disponibilité de séries de température et de données biologiques de terrain des espèces les plus représentatives de la communauté benthique sessile de la MNO, on étudie pour la première fois la relation entre la température et la mortalité à travers des modèles statistiques afin de générer un outil de prédiction des EMM.

L'ensemble des résultats de cette thèse a pour but d'offrir de l'information qui aide à anticiper les possibles effets du changement climatique dans la communauté macrobenthique sessile de la MNO. Notre objectif est de contribuer à la prise de décisions pour la conservation et la gestion de ces communautés.

La thèse est organisée comme suit :

Dans le chapitre 2, à travers les séries de température à fréquence horaire, disponibles pour quatre régions de la MNO obtenues au cours de la période qui va de 1999 à 2006 (période qui inclut les deux EMM les plus importants jamais observés dans cette région: 1999 et 2003; ainsi qu'un épisode mineur tant du point de vue de l'échelle spatiale que de l'intensité : 2006), on réalise une analyse descriptive détaillée par le biais de plusieurs descripteurs. Après avoir identifié les caractéristiques thermiques permettant de différencier les années avec des EMM et les années sans EMM, on évalue si les différences dans les niveaux de stress thermique durant les EMM expliquent les différences dans les taux de mortalité observés dans les différentes régions et profondeurs ainsi qu'entre les différentes années où se sont produits les EMM. Parallèlement, à travers les caractéristiques thermiques des différentes régions et de toutes les données de thermotolérance disponibles jusqu'à présent, on identifie le degré de risque pour les différentes régions analysées d'être impactées par de futurs EMM. Ce chapitre a été publié dans PLoS ONE.

Dans le chapitre 3 on étudie expérimentalement la réponse à la température de populations de *P. clavata* provenant de trois régions de la MNO (toutes les régions qui font l'objet de l'étude de la thèse sauf Port-Cros). On étudie en outre au sein de chaque région la réponse à la température de populations provenant de deux profondeurs et pour l'une des régions on compare la réponse de trois populations provenant de la même profondeur. Finalement, on compare la réponse de populations collectées au cours de l'année dans trois stations afin d'évaluer des différences dans la réponse à la température de populations ayant différents statuts physiologiques. À partir des résultats obtenus, nous évaluons le niveau de vulnérabilité des différentes régions en fonction de la réponse des populations de *P. clavata* au stress thermique et nous discutons le futur de ces populations en tenant compte de la présence de colonies «thermorésistantes».

Dans le chapitre 4 on introduit en guise de révision une famille de modèles statistiques appelée *Machine Learning* et son applicabilité pour traiter certains problèmes en écologie. Ces modèles ont été choisis pour analyser la relation de la température et de la mortalité sur le terrain dans le chapitre 5.

Dans le chapitre 5, à partir des données de terrain de plusieurs années sur la température et la mortalité dans les régions étudiées, certains des modèles exposés dans le chapitre 4 sont utilisés pour étudier la relation de la température et de la mortalité et pour générer des prédictions sur de futurs EMM.

10. Caractère interdisciplinaire de cette thèse

Il faut souligner que cette thèse fait partie d'une action de recherche interdisciplinaire dont l'objectif est de comprendre la réponse des communautés benthiques rocheuses côtières de la MNO face au changement climatique. L'objectif final est d'apporter des informations significatives pour aider à la prise de décisions dans la gestion et la conservation de ces communautés. Cette thèse a été développée au sein de l'équipe «Impact du changement climatique sur la biodiversité marine» de l'UMR 6540 DIMAR du Centre d'Océanologie de Marseille et a surtout bénéficié du projet MedChange (www.medchange.org) financé par l'Agence nationale de la Recherche et des travaux qui furent lancés dans le cadre de ce projet. Parmi les principaux travaux, on signalera :

- Les thèses doctorales de J. B. Ledoux (2010) et de K. Mokhtar-Jamaï (2011) sur la structuration génétique des populations de *C. rubrum* et de *P. clavata* à différentes échelles spatiales de la MNO ainsi que la relation de la diversité génétique et la tolérance à la température (développées dans l'UMR 6540 DIMAR, Centre d'Océanologie de Marseille) (Ledoux et. al., 2010a, b ; Mokhtar-Jamaï et al., 2011) ;

- La thèse de Marie La Rivière sur la communauté bactérienne associée à *P. clavata* dans différentes conditions thermiques afin de détecter de possibles changements qualitatifs et quantitatifs en fonction des conditions de températures et de détecter la présence d'organismes pathogènes (développée dans l'UMR 6540 DIMAR, Centre d'Océanologie de Marseille) (Bally et Garrabou, 2007 ; La Rivière et al., 2010) ;

- L'acquisition de séries de température à haute résolution (www.t-mednet.org) et la quantification des impacts du changement climatique dans la conservation de la biodiversité marine des côtes rocheuses de la mer Méditerranée (www.medchange.org).

Temperature anomalies and mortality events in marine rocky benthic communities: insights on factors behind differential mortality impacts in the NW Mediterranean

CHAPITRE 2

Abstract

Two large-scale mass mortality events (MMEs) of unprecedented extent and severity affecting rocky benthic communities occurred during the summers of 1999 and 2003 along the coasts of the NW Mediterranean Sea. These mortality outbreaks were associated with positive thermal anomalies. In this study, we performed an analysis of inter-regional and inter-annual differences in temperature (T) conditions associated with MMEs of the red gorgonian *Paramuricea clavata* by analyzing high resolution T time series (hourly records for 3 to 8 years) from four regions of the NW Mediterranean with differing hydrological conditions and biological impacts. High resolution records allowed a detailed analysis using classical and new descriptors to characterize T anomalies. We were able to determine that the MMEs were triggered by two main types of positive thermal anomalies, with the first type being characterized by short periods (2 to 5 days) with high Mean T reaching more than 27°C in some regions and being associated with high intra-day and intra-period variability, while the second type of anomaly presented long duration (near one month) at warm T (24°C) with low intra-period variability. Inter-regional patterns arose; some regions displayed both types of anomalies, while others exhibited only one type. The results showed that T conditions should be considered as the main factor that explains the observed inter-regional and inter-annual differences in mortality impacts. In explaining these differences, the late timing of T anomalies, in addition to their magnitude was found to be determinant. Finally, by combining thermotolerance experimental data with the maximal T stress conditions observed in the four regions, we were able to determine the differential risk of mass mortality across regions. We conclude that expanding high resolution T series is important for the development of sound management and conservation plans to protect Mediterranean marine biodiversity in the face of climate change.

Key-words: seawater temperature; positive thermal anomalies; mass mortality events; coastal rocky benthic community; NW Mediterranean; climate change.

1. Introduction

Coastal marine ecosystems harbor high biological diversity and are among the most productive systems in the world (Costanza, 1993; Harvell et al., 1999). These ecosystems are subjected to high levels of anthropogenic pressure, which could have serious implications for the well-being of societies dependent on these ecosystems for goods and services (Nicholls, 2007).

Overexploitation has been recognized as the major threat to marine ecosystems causing the decline of a number of target species and changes in the structure of food webs (Dayton, 1995; Jackson and Kidwell, 2001). However, pollution, invasive species, alteration and loss of habitats and, more recently, global climate change have also been reported to have significant effects in marine ecosystems (Steneck, 2001; Harvell and Ostfeld 2002; Hughes, 2003; Kappel, 2005; Harley et al., 2006).

The analysis of climate change impacts presents a unique challenge for conservation biology because they affect large spatial scales and because they are not easily alleviated by local management actions (Hughes, 2005). Likewise, these impacts affect most levels of biological organization: from population and life-history changes to shifts in the species composition and in the structure and function of ecosystems (Walther, 2002; Harley et al., 2006). Therefore, research efforts focused on providing meaningful data for the development of management plans are urgently needed to enhance the resilience of ecosystems facing current environmental changes (Hughes, 2003; McClanahan, 2007).

In the NW Mediterranean (NWM) Sea, recent studies have demonstrated a clear warming trend during the last century and the enhancement of stratification conditions during summer periods in the last 30 years (Romano and Lugrezi, 2007; Bensoussan, 2009; Coma et al., 2009; Romano et al., 2010; Vargas-Yáñez et al., 2010). In this region, warming has been found to be associated with shifts in species distributions (Francour et al., 1994; Bianchi, 2007) and mortality events observed during the last 30 years (Pérez et al., 2000; Garrabou et al., 2009). In particular, two recent large-scale (> 1000 km of coastline) mass mortality events (MME) of approximately 30 macro-benthic species including sponges, cnidarians, bivalves, ascidians and bryozoans, occurred during the summers of 1999 and 2003 along the coasts of Spain, France and Italy.

In 2006 and 2008, mortality events of a minor extent and severity were also documented in the NWM region (Bensoussan et al., 2010; Vezzulli et al., 2010; Huete-Stauffer et al., 2011).

All these events were associated with positive thermal anomalies (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009; Huete-Stauffer et al., 2011).

An analysis of the biological impacts of mentioned MMEs has revealed differential responses among species and their populations at all spatial scales considered (Garrabou et al., 2009). At the local level, colonies can show contrasting responses, ranging from severe to a complete absence of injuries. Within regions, populations can display low to high mortality, and there is a clear decrease of impact with depth (Linares et al., 2005). As an example, different red coral (*Corallium rubrum*) populations from the same region presented from 5% to 80% of affected colonies (Garrabou et al., 2001). This magnitude of differences has also been observed at the inter-regional level (Garrabou et al., 2009). Finally, the same regions affected by MMEs in different years exhibited differential impacts, both in magnitude and the depth range affected (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009).

In this study, we present an analysis of inter-regional and annual differences in T conditions associated with MMEs by analyzing high resolution T time series from four regions of the NW Mediterranean Sea with differing hydrological conditions (Bensoussan et al., 2010) and biological responses (Garrabou et al., 2009). The characterization of the thermal conditions of different regions and years that displayed mass mortality events and the analysis of corresponding impacts in the red gorgonian *Paramuricea clavata* populations, allowed for the first time the study of the relation of regional temperature conditions with observed impacts at the population level. High resolution T records allowed a detailed analysis using classical and new descriptors. Moreover, the results allowed the discussion of the potential role of temperature conditions and biological factors (e.g. acclimatization, local adaptation) that may underlie the differential impacts of the MMEs.

2. Materials and methods

2.1. Study area

The study was conducted in four locations of the NW Mediterranean basin (Fig. 1a), which were the following, from west to east: Parc Natural del Montgrí, Illes Medes i Baix Ter (L'Estartit, Spain); Riou (Marseille, France); Parc National de Port-Cros (France); and Reserve Naturelle de Scandola (Corsica, France) (Fig. 1b). These regions shared the common feature of NW Mediterranean waters of being characterized by a marked seasonality. From late autumn to winter (December-March), the seawater T slowly declines, reaching a minimum in March of approximately 13^{o}C before increasing slightly until the

formation of the thermocline (Margalef, 1985). Although they present similarity in their annual T cycles, during summer, the four regions present very distinct hydrographic conditions (Bensoussan et al., 2010). Riou exhibits the coldest conditions from depths of 5 to 40 m, while Scandola is the warmest site in its subsurface waters, and Medes and Port-Cros show intermediate conditions. From depths of 15 to 35 m, the warmest T occurs at Medes and Scandola, with Port-Cros being in an intermediate position (Bensoussan et al., 2010). With respect to the variability of the summer thermal regime, inter-regional differences are also observed. Riou is the most variable site from depths of 5 to 40 m because of the occurrence of upwelling, while Medes exhibits the highest variability at 40 m because of the recurrent downwelling. Finally, Port-Cros and Scandola display the maximum variability at 25-30 m because of oscillations of the thermocline that settles around these depths (Bensoussan et al., 2010).

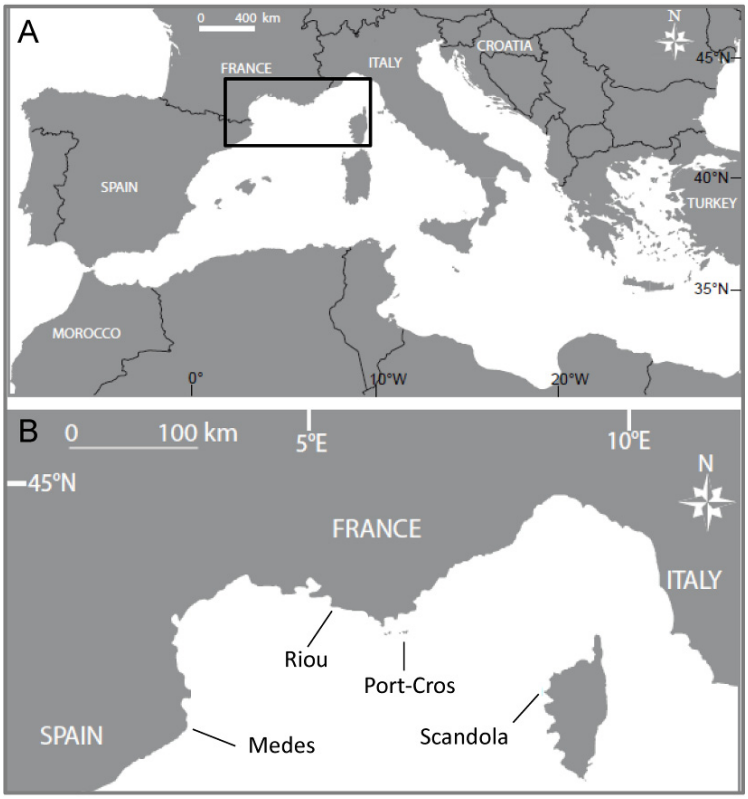


Figure 1. Northwestern Mediterranean Sea showing the limits of the study area (A) and detail of the NW Mediterranean with the four study regions (B).

2.2. Temperature measurement strategy

In each region, T records were registered by *in situ* Stowaway Tidbits autonomous sensors set up in sites exposed to dominant winds and currents.

The recorded period was from 1999 to 2006. Records began in June 1999 at Riou and Port-Cros, in July 2002 at Medes and in April 2004 at Scandola. Since 2004, measurements have been standardized at all regions to collect hourly records. Previously, T measurements had been recorded every 2 hours, and these time series were interpolated using an exact interpolation technique to obtain a set of synchronous hourly data. T data were not available for all years within each region; the available periods of T measurements at each depth are shown in Table 1. These temperature series were previously used to characterize temperature regimes in the four studied regions (Bensoussan et al., 2010).

Table 1. Available temperature data for the four study regions in the northwestern Mediterranean Sea at 10 and 25 m depths (*: for 2006, temperature at 15 m depth was analyzed instead of temperature at 10 m depth). The selected depths include the depth range in which the most severe impacts of MMEs were observed.

Geographic region	10 m	25 m
Parc Natural del Montgrí, Illes Medes i Baix Ter (L'Estartit, Spain)	2002-2006*	2002-2006
Riou (Marseille, France)	1999-2006	1999-2006
Parc National de Port-Cros (France)	1999, 2001 - 2006	1999-2000, 2002-2006
Reserve Naturelle de Scandola (Corsica, France)	2004-2006	2004 – 2006

2.3. Biological data collection

Biological surveys were conducted in the four study regions to investigate relationships between temperature conditions and population responses. For this purpose, we chose populations of the red gorgonian *Paramuricea clavata* as model species because the largest dataset was available for this species, and it was one of the most affected during the MMEs (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009). Furthermore *P. clavata* is considered one of the key species of Mediterranean coralligenous assemblages (Gili and Ballesteros, 1991; True, 1970). Since other macrobenthic species affected by the MMEs showed similar inter-regional and inter-annual pattern of mortality to *P. clavata*, the patterns obtained for this species may be considered representative of the MMEs impacts for other species as well (Pérez et al., 2000; Garrabou et al., 2009; authors unpublished data). For all

these reasons we contend that the use of *P. clavata* provide an excellent model for the analysis of the relationships between temperature conditions during temperature anomalies and biological impacts.

During the surveys, the percentage of recent tissue necrosis (i.e., denuded axis or recent epibiosis) was quantified in at least 100 colonies present at each site and depth surveyed. We considered a colony to be affected by mortality when it showed recent tissue necrosis over 10% of its surface. Finally, for each survey, the percentage of affected colonies was calculated as an indicator of the mortality impact (see Garrabou et al., 2009 for further information). Surveys were conducted on an annual basis at 2-7 sites within each study area. In cases where a MME was observed, the number of sites was increased when possible to better quantify the mass mortality impacts. More than 20.000 colonies were analyzed within the four study regions. We calculated the percentage of affected colonies within each region at every year that presented mass mortality events and inside each region we averaged the values of the years that not displayed mortality outbreaks. Percentages were calculated separately for 10 and 25 m depth ranges. In Port-Cros and Scandola, the surveys only concerned the 25 m depth because at 10 m, *P. clavata* populations are absent or show low abundance (Harmelin, 2004; Linares et al., 2005).

Kruskal-Wallis analysis was used to test for differences of mortality rates between all regions, years and depths. Multiple comparisons were studied through Mann-Whitney tests to determine specific differences between pairs of data. Nonparametric tests were selected because of the absence of normality and homoscedasticity in the dependent variable. The Kruskal-Wallis and Mann-Whitney tests were computed using PAST software (version 1.82b, Hammer et al., 2001).

2.4. Characterization of temperature anomalies related to mass mortality events

To characterize the temperature conditions of years associated with mortality events in each region, we combined classical and new descriptors to retain information on the magnitude, variability and duration of T anomalies, as well as the timing of the anomalies during the summer period (see below).

In the analysis, the period between 1st July and 30 September was arbitrarily considered as the summer period. Likewise, the depths considered were 10 and 25 m (12 and 24 m at Riou and Port-Cros in the 1999-2003 period, hereafter referred to as 10 and 25 m, respectively), which correspond to the suprathermoclinal and intermediate thermoclinal levels, respectively

(Bensoussan et al., 2010). We selected this period and these depths because the mass mortality events (MME) displayed the most severe impacts under these conditions (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009; Vezzulli et al., 2010).

Finally, we distinguished two types of years: those associated with mortality events in at least some of the studied regions (hereafter YMMEs), which included 1999, 2003 and 2006; and those presenting no mortality event signals (hereafter YNMMEs), which included 2000, 2001, 2002, 2004 and 2005. For all of the analyzed statistics, for each location and depth, each YMME was analyzed separately, while YNMMEs were studied together, averaging statistical values. Representation and analysis of the data were performed using SigmaPlot (version 10.0) and PAST (version 1.82b, Hammer et al., 2001) software, respectively.

2.4.1. Mean T, maximum T and coefficient of variation of the summer period

The Mean T, maximum T (Max T) and coefficient of variation (CV) were calculated to search for differences between YMMEs and YNMMEs and within YMMEs. The CV (summer standard deviation x 100/summer Mean T) was chosen as the measure of variability because it is the percentage of the degree of variability and can be interpreted independently from the mean.

2.4.2. Mean and CV of time intervals with the highest Mean T

Consecutive episodes of 2, 5, 10, 15, 30 and 40 days (taking 24 consecutive hours as a day, 48 consecutive hours for two days, and so on) with the highest Mean T were retained. For each year, region and depth, there was a unique corresponding consecutive period of a specific length with the highest Mean T. Thus, the value of this Mean T and the corresponding value of the CV of each length period were retained with the aim of capturing the T magnitude and associated variability of the hottest periods of short, intermediate and long duration.

2.4.3. Timing of periods with the highest Mean T

The timing of the above-mentioned 15- and 40-day periods was analyzed. Timing refers to the point in time in the summer when these periods occurred. For the 15-day episodes, summer was divided into 6 two-week summer periods: 1st-15 July, 16-31 July, 1st-15 August, 16-31 August, 1st-15 September and 16-30 September. Then, the 15-day episodes with highest Mean T were associated with the two-week period in which most of the episode

occurred. For the 40-day episodes, the summer period was divided into months: July, August and September. Again, the 40-day episodes with highest Mean T were associated with the month in which most of the episode occurred. By analyzing the timing of the temperature anomalies, we intended to explore the response of affected species to similar temperature stresses occurring at different times during the summer.

2.5. Ordination of YMMEs and YNMMEs through T statistics

The analyzed statistics (except those on temperature anomalies timing) were ordered through Principal Component Analysis (PCA) with the aim of synthesizing the information provided by the different T indicators. Two analyses were performed, one for the 10 m depth and another for the 25 m depth. In addition to the analyzed statistics described above, three further variables were considered to perform the PCA. We included, in one side, the total duration (as the proportion of summer time) inside the [24-25[^{o}C T class and the longest consecutive duration inside this class. These statistics were considered since it was documented that during 1999 summer, long duration near 24^{o}C occurred at Riou and at Port-Cros (Harmelin, 2004; Bensoussan et al., 2010). In the other side we included the average CV of the 5 hottest summer days. It was calculated averaging the CV value corresponding to the 5 summer days with the highest Mean T. This indicator was included with the aim of considering variability of the thermal regime at shorter time scales (e.g. inside days). Overall, a total of 15 variables were available to perform the analyses, but because of the very high correlation between some of them (Pearson correlation coefficient > 0.8), redundant variables were removed to perform PCAs. Finally, a total of six and eight variables were retained to perform the 10 and 25 m depth PCA, respectively. For the 10 m depth PCA the retained variables were the Mean T of the 5 and the 40 consecutive days with highest mean T (Mean_T_5 and Mean_T_40 respectively), the CV of the 5 and the 40 consecutive days with highest mean T (CV_5 and CV_40 respectively), the average CV of the 5 hottest summer days (Mean_CV_5) and the total duration inside the [24-25[^{o}C T class (Dur_24). For the 25 m depth PCA all the variables retained for the 10 m depth PCA, the CV of the 15 consecutive days with highest mean T (CV_15) and the longest consecutive duration inside de [24-25[^{o}C T class (Max_cons_dur_24) were analyzed.

As for the previously calculated statistics, YNMMEs were considered together, while YMMEs were studied separately.

2.6. Confronting thermotolerance experiment results and field T conditions

The available information from the experimental results on the thermotolerance of NWM rocky benthic species (Table 2) was contrasted with the most severe field T conditions observed in the four studied regions. An inverse second-order regression ($f=y0+(a/x)+(b/x^2)$) was fitted to the Mean T of increasing time intervals (from 2 to 40 days) with the highest Mean T with the aim of obtaining a domain of possible conditions in each region given the available datasets and comparing them with the upper thermotolerance limits from experimental data. Given that points located in domains below the regression curves indicate species at risk under actual conditions, we suggest that differences with respect to the distribution of experimental data above and below the regression curve could provide clues related to the differential risk of mortality among regions.

Table 2. Data from previous experimental work on T effects on mortality (necrosis) of the NWM rocky benthic species used to produce Figure 7.

Species	Tested T (ºC)	References
Cladocora caespitosa	24, 26	(Rodolfo-Metalpa et al., 2006)
Corallium rubrum	24, 25, 27	(Torrents et al, 2008; Previati et al., 2010)
Eunicella singularis	*24*	*(Ferrier-Pages et al., 2009)*
Oculina patagonica	24, 26	(Rodolfo-Metalpa et al., 2006)
Paramuricea clavata	23, 24, 25, 25, 27	(Bally and Garrabou, 2007; Coma et al., 2009; Previati et al., 2010, Chapter 3)

3. Results

3.1. Biological data

Clear differences were found in the mortality rates associated with different years, regions and depths, and these differences were significant (Kruskal-Wallis p-value<0.05). The highest mortality rates were found at Riou in 1999 at 10 and 25 m depths, at Riou in 2003 at the 10 m depth and at Port-Cros in 1999 at the 25 m depth, which all presented between 23 and 46% affected colonies (Fig. 2). Multi-comparison analyses did not indicate significant differences among these observations (p-value >0.05). These comparisons also showed that the cases with high mortality rates presented significant differences compared with all remaining cases, which experienced low to zero mortality rates (p-value<0.05). However, there was an exception for Riou in 2006 at the 10 m depth, which did not present differences with Port-Cros in 1999 at the 25 m depth. At this time and depth, Riou presented nearly 10% affected colonies, which was a value that was significantly higher than those of observations associated with low mortality rates (p-value<0.05, Fig. 2).

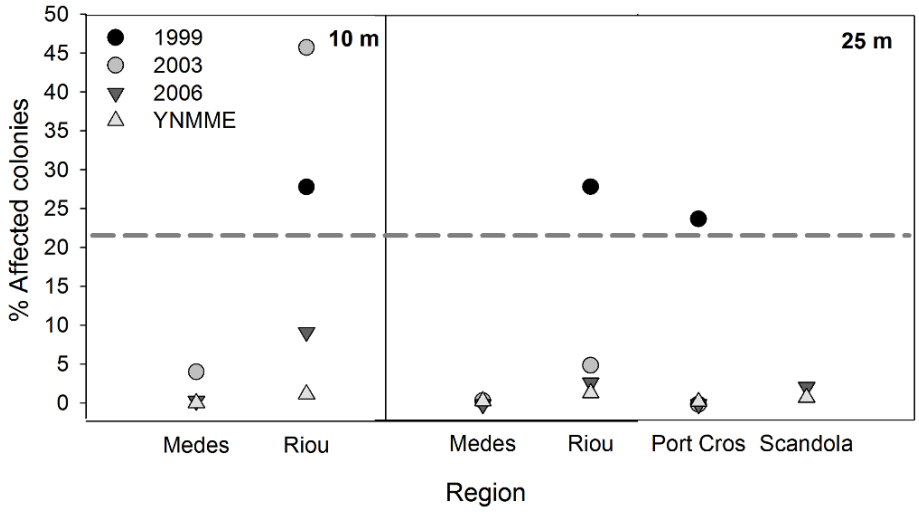


Figure 2. Percentage of affected colonies with ≥ 10% of the colony surface showing recent necrosis (denuded axis or recent epibiosis) for *Paramuricea clavata* populations in the four study regions of the northwestern Mediterranean sea at 10 and 25 m depths during 1999, 2003, 2006 and YNMMEs. The cases with the highest rates of necrosis are those above the grey dashed line. Within this group of data there were no statistical differences in mortality rates (p-value>0.05), while % of affected colonies showed statistical differences with the remaining years, regions and depths (p-value<0.5). There was an exception for Port-Cros in 1999 at the 25 m depth, which did not present differences with Riou in 2006 at the 10 m depth.

3.2. Characterization of the summer thermal regime of YMMEs

3.2.1. Mean T, maximum T and coefficient of variation of the summer period

At 10 m depths, the highest summer Mean T was observed in 1999 at Riou and Port-Cros and in 2003 at Medes (Table 3). For this year at these regions, the highest Mean T values were more than 1°C warmer than those in all remaining years. In particular, this value for Riou in 1999 was almost 2.5°C warmer than for YNMMEs. Finally, Scandola did not present important differences between 2006 (the only YMME with T data available) and YNMMEs. The Max T was reached in 2003 at Medes, Riou and Port-Cros and in 2006 at Scandola. In this last region and year, the highest Max T of all regions and years was observed, reaching almost 28°C (Table 3). Additionally, either 2003 or 2006 was the year that presented the highest CV in all regions. The high values of the CV in Riou are remarkable in both YMMEs and YNMMEs in comparison with the other regions (Table 3).

At the 25 m depth, as at the 10 m depth, the highest Mean T in Riou and Port-Cros were found in 1999 (1 to 2°C higher than other years). No remarkable differences were found among years for the other regions. However, in relation with the Max T, clear differences were found between Medes in 2003 and 2006 compared with YNMMEs (up to 2°C warmer) and Port-Cros in 1999 compared with all other years (1.3°C warmer). In contrast, in Scandola in 2006, the Max T was 1°C colder than in YNMMEs, indicating enhanced stratification, with warm T limited to shallow depths at this location. In Medes, 2003 and 2006 were more variable than YNMMEs, while 1999 and 2003 presented higher CVs than 2006 and YNMMEs in Port-Cros (Table 3).

Table 3. Mean T, maximum T (Max T) and coefficient of variation (CV) for the summer period of the four study regions for 1999, 2003, 2006 and YNMMEs (mean ± SD) at 10 and 25 m depths.

Region	Year	Mean summer T (°C)	Max. summer T (°C)	Summer CV (%)	Mean summer T (°C)	Max. summer T (°C)	Summer CV (%)
			10 m depth			25 m depth	
Medes	2003	22.3	25.5	8.4	18.3	24.5	15.2
	2006	21.6	25.0	10.1	19.6	24.9	14.3
	YNMME	21.2 ± 0.7	23.4 ± 1.1	4.6 ± 1.4	19.5 ± 0.3	22.9 ± 0.9	9.9 ± 3.3
Riou	1999	21.4	25.0	15.1	19.4	24.1	17.4
	2003	20.3	27.6	17.7	17.5	24.2	13.4
	2006	20.2	26.8	16.2	18.3	23.6	15.0
	YNMME	19.0 ± 0.3	24.7 ± 0.9	17.5 ± 1.2	17.5 ± 0.3	23.8 ± 1.3	15.9 ± 2.4
Port-Cros	1999	22.8	25.5	8.3	20.3	25.1	15.0
	2003	21.7	27.2	12.8	18.1	23.0	13.6
	2006	22.0	26.5	8.8	18.9	23.1	9.3
	YNMME	21.4 ± 0.9	24.9 ± 0.8	8.9 ± 1.6	18.4 ± 0.6	23.8 ± 0.4	12.1 ± 1.1
Scandola	2006	23.0	27.9	7.0	19.7	23.8	9.3
	YNMME	23.3 ± 0.4	25.7 ± 0.5	4.8 ± 1.3	19.9 ± 0.7	24.8 ± 0.5	12.0 ± 0.3

3.2.2. Mean T and CV of consecutive time intervals with the highest Mean T

In general, at the 10 m depth, YMMEs presented a higher Mean T than YNMMEs, regardless of the time interval considered (Figs 3a-d). However, inter-annual and inter-regional differences were observed. Medes in 2003 and 2006 (Fig. 3a) and Riou and Port-Cros in 1999 (Figs. 3b and 3c) presented remarkable constancy throughout all time periods considered compared with YNMMEs, reaching a Mean T near 24°C in the longest periods and a T near 25°C in the shorter periods considered. On the other hand, clear differences with YNMMEs were also found in the Mean T for short periods at Medes and Riou in 2003 and 2006 and at Scandola in 2006, where the Mean T values recorded were approximately 2-3 ºC higher than the values found in YNMMEs (Figs. 3a, 3b and 3d).

Changes in the CV for the different time intervals were also observed during YMMEs at 10 m depth (Figs. 3e-h). In Riou and Port-Cros in 1999, the constancy in the Mean T over the different time intervals was reflected in a drastic decrease in the CVs compared with other years (10 and 5.5%, respectively, for the longest episodes) (Figs. 3f and 3g). For the other

investigated years, Riou and Port-Cros showed CV values similar to those for YNMMEs, except in Port-Cros in 2003, where the CV for short periods was greater than for YNMMEs. In Medes and Scandola, YMMEs were characterized by an increase of the CV, especially when long periods of time were considered (Figs. 3e and 3h).

At the 25 m depth, the Mean T of YMMEs versus YNMMEs did not differ as strongly, and it was warmer for all time periods examined only in Medes in 2006 and Riou and Port-Cros in 1999 (Figs 4a-c). Medes in 2003 presented a higher T than YNMMEs, but only for short-length (≤5 days) episodes (Fig. 4a). All other years and regions did not present remarkable differences with YNMMEs (Figs. 4a-d). The most important differences compared with YNMMEs (2 to 4°C) were observed in Port-Cros in 1999, which presented a notable constancy in exhibiting a relatively high T throughout all time periods considered, reaching Mean T from 23.5°C to 24.5°C for the longest and the shortest periods, respectively (Fig. 4c). A similar pattern, though mainly concerning intermediate and long episodes, was observed in Riou in 1999 (Fig. 4b).

Because of the relative constancy in the 1999 Riou and Port-Cros thermal conditions, the CV was lower than that in all remaining years during intermediate and long episodes in Riou (Fig. 4f) and for long episodes in Port-Cros (Fig. 4g). The remaining years and regions did not differ significantly from YNMMEs (Figs. 4e-h).

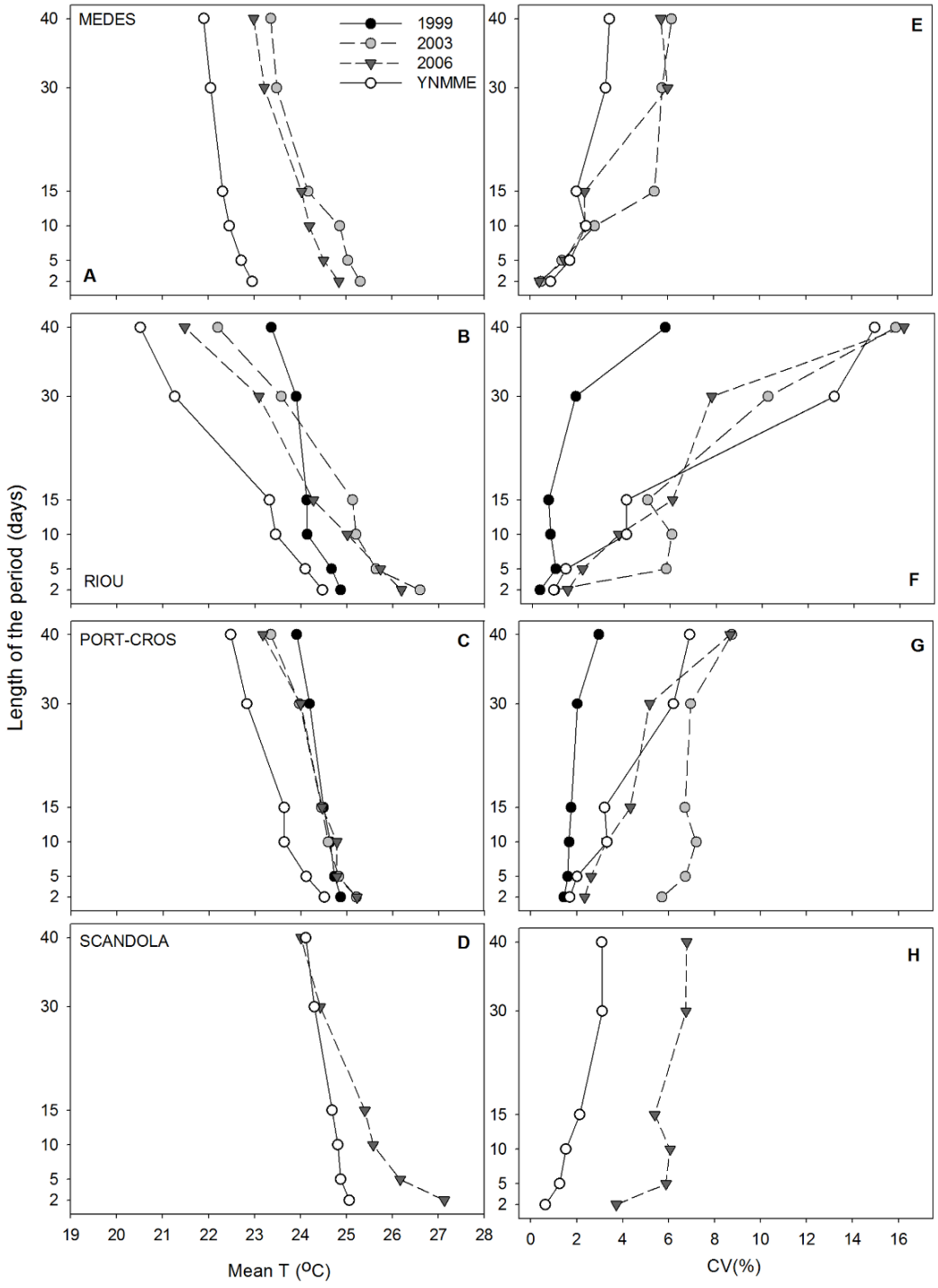


Figure 3. Mean temperature (°C) and coefficient of variation (%) of consecutive episodes of 2, 5, 10, 15, 30 and 40 days with the highest mean temperature. Data are presented for the four study locations at 10 m depth.

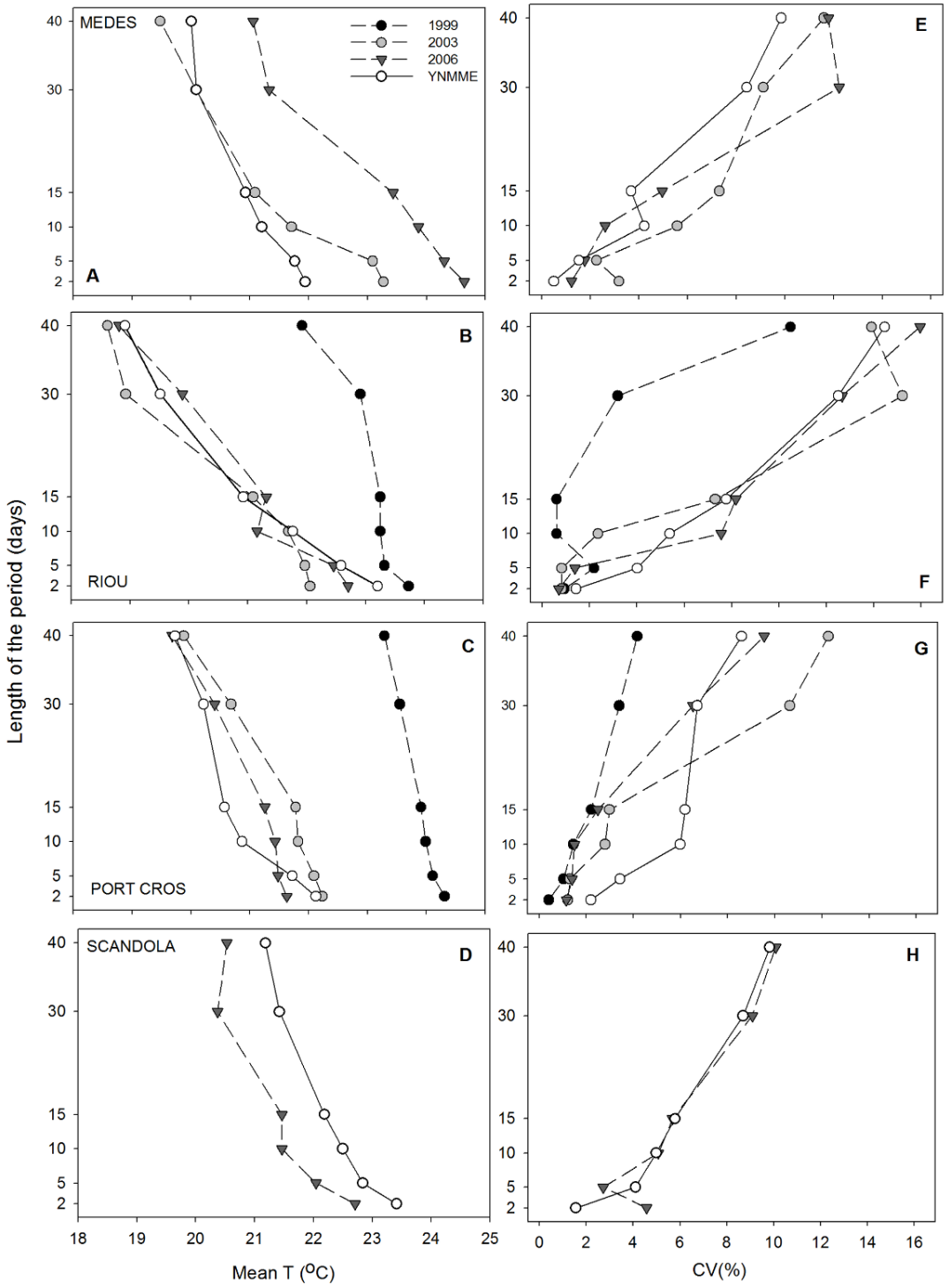


Figure 4. Mean temperature (°C) and coefficient of variation (%) of consecutive episodes of 2, 5, 10, 15, 30 and 40 days with the highest mean temperature. Data are presented for the four study locations at 25 m depth.

3.2.3. Timing of consecutive time intervals with the highest Mean T

At the 10 m depth, the 15-day episodes with the highest Mean T were relatively well segregated according to years (Fig. 5a). During 2006, these episodes occurred between the beginning and the middle of summer (16-31 July in Riou and Port-Cros and 1st to 15 August in Medes and Scandola). In 2003, they occurred mainly in the middle of the summer period (1st to 15 August in Riou and 16 to 31 August in Medes and Port-Cros), and in 1999, they occurred late in the summer period, between 1st and 15 September (Fig. 5a). For YNMMEs, the warmest 15-day consecutive episodes occurred in August, though there was a great deal of variability depending on region (Fig. 5a).

When 40-day consecutive episodes were considered, 1999, 2003 and 2006 were again well segregated. These episodes occurred in July during 2006, in August during 2003 and in September during 1999. During YNMMEs, they were mainly distributed in the middle of the summer, although great variability was observed within some regions (Fig. 5b).

At 25 m, both 15- and 40-day episodes occurred first in Medes 2006 (between 1st and 15 August and in July, respectively), while for all other YMMEs, episodes of both lengths occurred during September (Figs. 5c and 5d). YNMMEs presented intermediate and long hottest episodes, mostly at the end of the summer and always earlier than for YMMEs, although variability within some regions was observed (Figs. 5c and 5d).

3.3. Ordination of YMMEs and YNMMEs through T statistics

Considering the PCA for the 10 m depth, the first two axes accounted for 77% of the variance of the data. The first axis, which retained 42% of the variance, was useful for discriminating two main types of T anomalies. Riou and Port-Cros in 2003 and Scandola and Riou in 2006 were positively associated with this axis (Fig. 6a). Projection of the original T variables illustrates the summer thermal characteristics of these regions and years, which were associated with high T during short periods of time (five days) and with large hourly variability during the hottest summer days. High variability within short, intermediate and long hottest episodes was also a feature of these years. Negatively associations with the first axis were found for Riou and Port-Cros in 1999 and, with lower associated scores, Scandola in YNMMEs and Medes in 2003 and 2006. With the exception of Scandola in YNMMEs, these years represent a second type of thermal anomaly. This type of anomaly was characterized by long total and consecutive durations during warm T as well as by low variability at all time scales considered (Fig 6a).

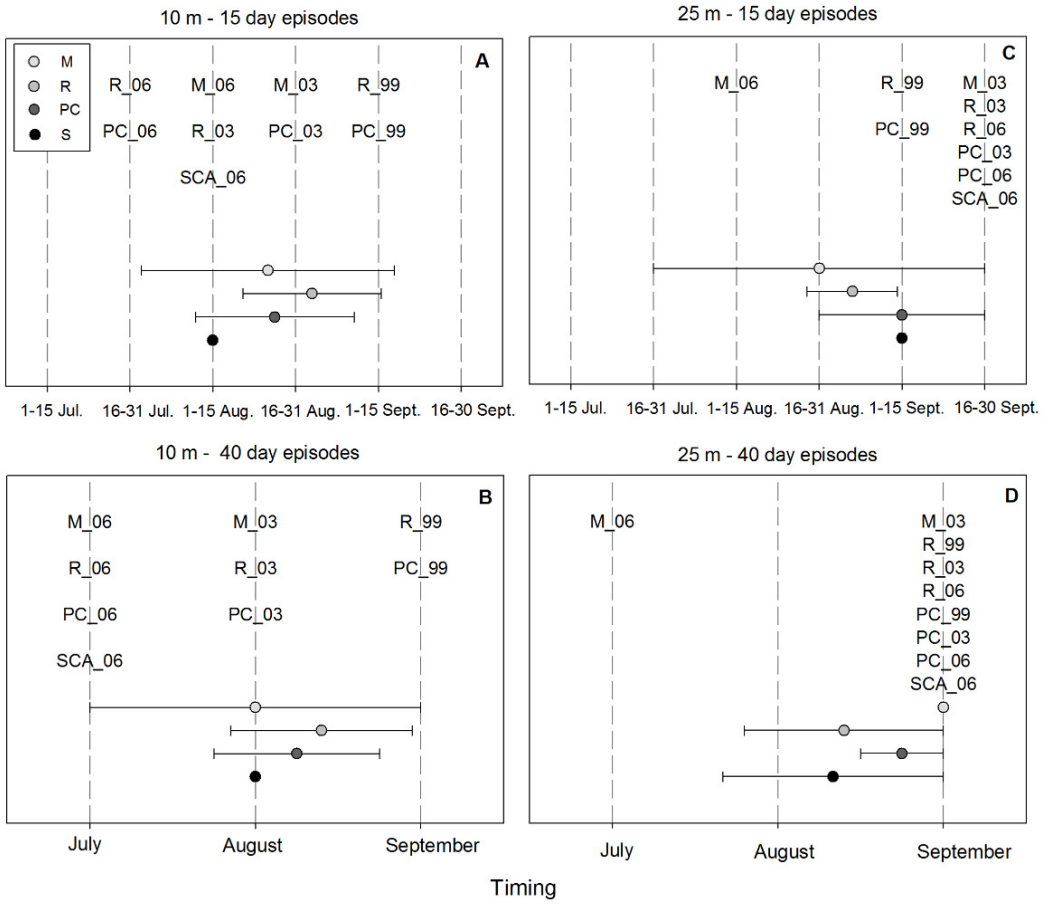


Figure 5. Timing of consecutive episodes of 15 (A, C) and 40 days (B, D) with the highest mean T for 10 and 25 m depths. The average ± SD is presented for YNMMEs. Cases were ordered in the Y axis to facilitate data reading, but their position in this axis does not provide any information. M: Medes, R: Riou: PC: Port-Cros and S: Scandola.

The position of Scandola YNMMEs could be explained because the subsurface waters of this region are the hottest among the four regions (Table 3).The second axis accounted for the characteristics of YNMMEs (with the exception of Scandola YMMEs), which were mainly low Mean T of short and large consecutive episodes with the highest Mean T (Fig. 6a).

At the 25 m depth, the first two axes accounted for 71% of the variance of the data (Fig. 6b). The first axis (51% of variability) distinguished the years that presented anomalous thermal regimes from those that did not. These years were 1999 for Port-Cros and Riou and 2006 for Medes and were characterized by a relatively long duration within warm T and relatively high T in short and long intervals with the highest Mean T. The second axis explained low data variability and did not clearly segregate YMMEs from YNMMEs. It was associated with variability related to different length episodes and separated regions inside YMMEs and YNMMEs (Fig. 6b).

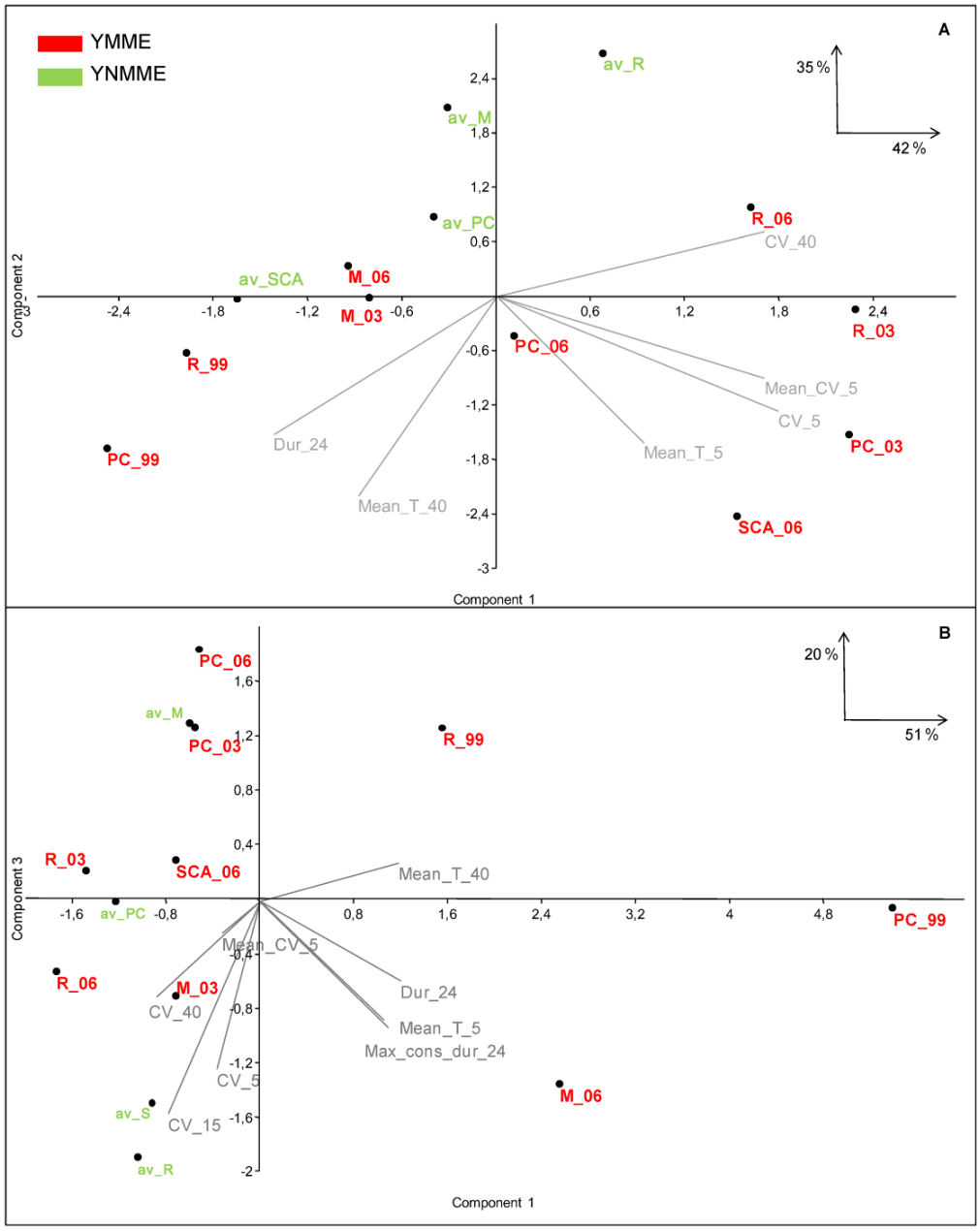


Figure 6. Representation of the first two axes of the Principal Component Analysis using T data from 10 (a) and 25 m depths (b). Two first axes were retained at both depths, accounting for 77 and 71 % of data variability. The original T variables used to perform the analyses are represented in grey. Scandola in 2004 at the 10 m depth was not considered among the Scandola YNMMEs in the PCA because this location and year presented the particularity of reaching high temperatures (but not as high as for YMMEs at this location) with no mortality, so it affected the data ordination when considering all locations together. M: Medes, R: Riou: PC: Port-Cros and S: Scandola.

3.4. Confronting thermotolerance experiment results and field T conditions

The distribution of the experimental thermotolerance data points around the field T data curves provided clues related to the degree of vulnerability of the different species under recent T conditions in the 4 regions (Fig. 7). The experimental results indicated that short to moderate exposure (1 to 14 days) to 25°C and short exposure to 26 and 27°C (1 to 3 days) could lead to mortality (with the exception of the symbiotic species *C. caespitosa* and *O. patagonica*, which seem to be more resistant to these T conditions).These temperature conditions were attenuated in Medes and Port-Cros regarding exposure to 25°C, and there was very low or nonexistent exposure to 26 and 27°C at these sites. Conversely, these conditions were more frequent in Riou and Scandola for YMMEs, reaching longer durations at 25 and 26°C and, in the case of Scandola, also at 27°C (Fig. 7). Therefore, Medes and Port-Cros appear to represent the less risky of the investigated regions, while in contrast, Riou and Scandola appear to be the most risky regions in terms of higher chances of experiencing mortality.

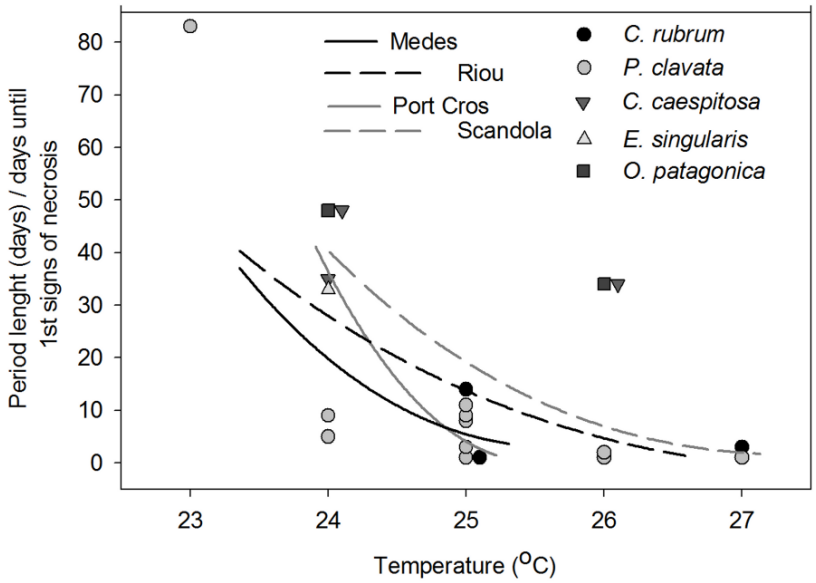


Figure 7. Inverse second order regression (f=y0+(a/x)+(b/x^2) fitted to field T data (highest mean T of different length episodes) of the four study regions at 10 m depth (complete and dashed lines) and experimental results (day of 1st signs of necrosis) from different rocky benthic species (grey and black symbols) obtained from the bibliography and the authors' unpublished data (see Table 2). For *E. singularis* and *O. patagonica* at 24 °C and for *O. patagonica* at 26 °C, the number of days until the 1st signs of necrosis actually indicates the number of experimental days since no necrosis was observed until the last day. The aim of the figure is to represent a composite function over which, under experimental conditions, the species seems to tolerate field T regimes and under which species are affected by tissue necrosis.

4. Discussion

In this study, we addressed for the first time the relationship of inter-regional and inter-annual differences in temperature conditions with the observed impacts in the macrobenthic populations of the NWM Sea during mass mortality outbreaks.

We were able to recognize two types of thermal anomalies that were likely to trigger an MME. The first type was characterized by short episodes (2-5 days of duration) with high Mean T, which were near 27°C in some regions, and high intra-day and intra-period (2-5 days) variability. The second type of anomaly presented long periods (30-40 days) with warm Mean T of approximately 24°C associated with low intra-period variability.

We found inter-regional and inter-annual differences in the occurrence and characteristics of the recorded anomalies. Riou and Port-Cros showed both types of anomalies: long term in 1999 and short term in 2003 and 2006. Furthermore, Medes displayed only long-term anomalies in both years in which anomalies were analyzed (2003 and 2006). Finally, in Scandola, a short-term anomaly was observed in the only year with anomalous T conditions (2006) for which data were available. It is worth noting that both in 2003 and in 2006, the two types of anomalies occurred simultaneously among regions, indicating that two of the largest heat-waves to ever peak over southern Europe (Schär, 2004; Rebetez et al., 2009) resulted in differential anomalous warming conditions in the water column. These differences could be attributed to the distinct summer hydrological conditions found in the four study areas (Bensoussan et al., 2010).

In Medes, there are recurrent downwellings (>40 m) during summer (Bensoussan et al., 2010), which carries increased T further down in the water column, resulting in warm conditions over longer periods, but never reaching the elevated temperatures found in other study areas. In Riou and Port-Cros, the hydrological conditions can experience abrupt changes under the influence of Mistral (NWN) winds (Millot, 1979). The lower frequency and shorter duration of these winds prevent the upwelling of deep, cold waters and the subsequent breakage of the thermocline, thus allowing the T to increase (Bensoussan et al., 2010). Scandola exhibits stable summer stratification conditions (Bensoussan et al., 2010), and thus, the high air temperature and calm weather conditions recorded during the 2006 heat wave resulted in a significant T increase at the suprathermoclinal level. The occurrence of the long- and short-term anomalies recorded can likely be mainly attributed to the timing of calm conditions in the early-middle for 2003 and 2006, and late summer for 1999. The observed differences in the magnitude of both types of T anomalies were able to explain the differences in mortality rates observed in the field. Short periods of high T and long periods

of warm T were associated with high mortality rates, being the attenuation of these characteristics (short periods of moderate T or shorter periods of warm T) linked to a significant decrease of mortality rates. Finally, years without thermal anomalies presented negligible mortality rates. Despite these relationships, it is important to precise that within the same region populations experiencing the same thermal regime presented different mortality impacts during MME (Garrabou et al., 2009; Garrabou et al., 2001), indicating, thus, that other factors than T may be involved in modulating the mortality impacts (see below).

The temperature conditions associated with T anomalies may be related to different biological mechanisms resulting in the death (total or partial) of organisms. In relation to long-term anomalies, the highest mortality rates were observed when long periods of warm Ts occurred at the end of the summer. Mediterranean suspension feeder species exhibit energetic constrains during summer (Coma and Ribes, 2003), since these organisms have to cope with high respiration demands because of warm T during a period of food scarcity (Coma et al., 2009). When these conditions are prolonged, as in the case of the years with anomalies, the organisms can suffer physiological stress that can ultimately lead to partial or total death of some specimens (Coma et al., 2009). In previous studies, experimental data demonstrated that long duration exposure to warm T (~ 23^{o}C for >40 days), similar to the conditions observed during long-term anomalies, could cause the appearance of the firsts signs of necrosis (Coma et al., 2009). In the same experiment, when the colonies were fed, the time of exposure to warm T before observing necrosis almost doubled, clearly indicating that feeding helps to cope with physiological stress (Coma et al., 2009). Therefore, the physiological status of organisms is important in modulating their response to thermal stress. This factor may be behind the observed differential mortality observed during long-term anomalies in Riou and Port-Cros in 1999 and Medes (2003 and 2006).

The high T observed during short-term anomalies reached lethal levels for the benthic species of the study regions, as demonstrated through thermotolerance experiments with different Mediterranean gorgonian species (Bally and Garrabou 2007; Torrents et al., 2008; Previati et al., 2010; Chapter 3). Additionally, the high intra-day and intra-period variability that characterized this type of anomaly could be an additional stress factor on the organisms, as found in some tropical coral species (Coles, 1975; Sammarco, 2006). Nevertheless, when this type of anomaly occurred at the beginning of summer, such as those that took place in 2006, they resulted in less severe impacts on these populations, probably because the species were less affected by energetic constraints during this period (Coma et al., 2009).

Finally, for both types of T anomalies, the induction of mortality being provoked by thermo-dependent pathogens cannot be discarded, considering that experimental and field data demonstrated that Ts ≥ 22°C promote pathogen virulence and/or increase host susceptibility (Bally and Garrabou, 2007; Vezzulli et al., 2010).

Overall, the results of this study indicated that inter-regional differences in mortality rates should be mainly attributed to differences in the T conditions recorded in each region and year with a T anomaly. However, because the populations inhabiting the studied regions were subjected to different magnitudes and timing of T anomalies, we could not determine the potential role of biological factors, such as acclimatization (Wilson and Frankling, 2002), local adaptation (Williams, 1966; Kawecki and Ebert, 2004) or even contemporary evolution (Hendry and Kinnison, 1999), previously highlighted for other marine species (Smith-Keune and Oppen, 2006; Baums, 2008; Oliver and Palumbi, 2011). Bearing in mind that most of the species affected by the MME inhabit different thermal regimes within the NW Mediterranean basin (Margalef, 1985; Bensoussan et al., 2010) and appear to be characterized by significant genetic differentiation, even at reduced spatial scales of several meters (Duran et al., 2004; Ledoux et al., 2010a), it seems likely that selective processes could play a role in determining their response to T anomalies. The available experimental data suggest an inter-depth differential response to the same experimental T (Torrents et al., 2008; Ledoux et al., 2010b). To further explore the role of selective processes in these phenomena, regional-scale thermotolerance experiments should be conducted.

The combination of field T data on the most extreme observed Ts of each studied region with available thermotolerance data on NW Mediterranean anthozoan species allowed us to assess the risk of suffering MMEs in the different study regions (Fig. 7). In general, the T conditions observed in Medes, which exhibits an absence of extreme T (short-term anomaly) and attenuated characteristics of long-term anomalies, do not reach values causing severe damages to these organisms. Therefore, this region could be associated with a lower risk of mortality outbreaks compared with the other regions under present T conditions. In contrast, Riou and Scandola appeared to be the regions with the highest risk, as in both cases, the anomalous T conditions reached values beyond the tolerance of the species addressed in this study. The case of Riou is interesting because it is located in one of the coldest areas of the NW Mediterranean (Antonov et al., 1998.). The fact that both types of anomalies could occur in this area, combined with the shallow distribution of species affected by MMEs (Pérez et al., 2000; Garrabou et al., 2009) leads to an unfavorable scenario for Riou populations in the future. Scandola presented the most extreme T of short and intermediate-length periods, which makes it difficult for species affected by MMEs in shallow depth ranges

to survive in this region. In fact, the absence of *P. clavata* populations at the 10 m depth in this area could indirectly suggest that T could be modulating this species' depth distribution, although effects of other environmental factors cannot be discarded (light, water motion, food availability) (Zabala and Ballesteros, 1989; Garrabou et al., 2002). Finally, Port-Cros occupied an intermediate position in terms of the risk of presenting MMEs, mainly because of the absence of high T episodes. In accordance with these results, the number of species affected and the incidence of mortality during MMEs showed the lowest values in Medes, followed by Port-Cros and, finally, by Riou and Scandola (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009).

In this study, we demonstrated the utility of acquiring and analyzing high resolution T series, which allowed us to determine the main T conditions responsible for the differential mortality impacts observed in the NWM basin and to assess the risk of MMEs in the studied regions. The acquisition of new high resolution T time series in different regions of the Mediterranean (e.g., /T-MEDNet, http://t-mednet.org) will allow expanding the analysis to better characterize and understand current shifts in environmental conditions at larger spatial scales. Additionally, under the present warming scenario for the Mediterranean area (Somot et al., 2008), these data will be key components in the development of MME risk maps at the scale of the NW Mediterranean. This information is urgently needed to develop sound management and conservation strategies to face the impacts of climate change on the rich marine biodiversity in the Mediterranean region (Coll et al., 2010).

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Thermotolerance experiments with *Paramuricea clavata* populations at the NW Mediterranean scale: a step forward to better understand the response of rocky benthic populations to recent thermal anomalies

CHAPITRE 3

Abstract

The occurrence of recent mass mortality events in the NW Mediterranean (NWM) coastal waters associated with sea water temperature anomalies raised the interest on rocky benthic species thermotolerance. Thermotolerance features provide basic information to explore the impacts of warming in marine ecosystems. However a more comprehensive view should take into account potential differential responses across populations at different spatial scales. This seems reasonable if we consider that in the analysis of impacts of disturbance linked to anomalous temperatures, high levels of variability were commonly observed raising the question of which factors and mechanism could be behind these differences. In this study we carried out a thermotolerance study with populations of the red gorgonian *Paramuricea clavata* from the NWM region dwelling in distinct temperature regimes (different regions and depths) with the aim to provide basic information on thermotolerance features of the species and to determine potential thermotolerance differences of populations dwelling in contrasted thermal regimes. The results suggested that 25^{o}C is a critical temperature for *P. clavata* since after some days of exposition to this T, firsts signs of necrosis appeared despite of the origin of the populations. Higher temperatures as 27 and 28^{o}C were lethal for the species because all studies populations showed signs of necrosis after very short periods of exposure. The thermotolerance of populations dwelling in different thermal regimes (inter-regional and inter-depth analysis) did not differ importantly. In fact, different populations living in the same region and depth accounted for the greatest variability in the response to temperature which could be indicating the presence of adaptative processes to local conditions other than temperature. The combination of thermotolerance and field temperature data suggested that shallow populations of *P. clavata* are living near their upper thermal limit. Despite this, the presence of relatively high proportion of resistant colonies at the end of the experiments in most of the studied populations could be associated with the capacity to acclimatize and or adapt to future warmer conditions. Overall, performed experiments provided important clues on the effect on warming in NWM *P. clavata* populations. Expanding these studies to other species and regions is of great interest to generate reliable tools to anticipate the effects of climate change in the NWM coastal rocky benthic communities.

Key-words: climate change; *Paramuricea clavata*; thermotolerance; acclimatization/adaptation; mass mortality events; NW Mediterranean.

1. Introduction

Increase in seawater temperature is one the major effects of global climate warming (IPCC, 2007). Despite the complexity of the biological responses to environmental changes (Harley et al., 2006), the increase of mass mortality events and diseases in marine species worldwide has been clearly linked to the ocean's warming with particular emphasis on coastal and tropical species (Harvell et al., 1999, 2002; Hughes et al., 2003). In the NW Mediterranean (NWM) area, during the summers of 1999 and 2003, mass mortality events of unprecedented severity affected marine coastal (0 - 45 m) rocky benthic communities over large spatial scales (several thousand kilometers of coastline) involving Cnidarians and other metazoan such as Porifera, Mollusca, Crustacea, Echinodermata, Bryozoa and Ascideacea (Cerrano et al., 2000; Pérez et al., 2000; Garrabou et al., 2009; Cebrian et al. 2011). These events which displayed great variability in mortality rates, from individual to regional scales (Perez et al., 2000; Garrabou et al., 2001; Garrabou et al., 2009) have been related to positive temperature anomalies (Garrabou et al., 2009, Bensoussan et al., 2010; Cebrian et al., 2011) associated with the current NWM warming (Bensoussan et al., 2009; Romano and Lugrezi, 2007; Vargas-Yañez et al., 2010).

The link of mortality events with abnormal seawater temperatures has raised the concern on species thermotolerance because it may provide crucial knowledge to the understanding of the effects of warming on marine organisms. Special interest has been devoted to the study of tropical corals species (e.g. Hoegh-Guldberg and Smith, 1989; Lesser et al., 1990; Lesser, 1997; Coles and Brown, 2003; Oliver and Palumbi, 2011). However, only a few number of studies have dealt with the species response to temperature in temperate systems, and particularly in Mediterranean ones (Rodolfo-Metalpa et al., 2005, 2006a, 2006b and 2008; Torrents et al., 2008, Coma et al. 2009; Ferrier-Pages et al., 2009; Previati et al., 2010; Pey et al., 2011) despite the recurrent occurrence of the above mentioned events since 1999. Previous works on the thermotolerance of Mediterranean anthozoan species showed that thermal stress affects growth and survival of the symbiotic species *Cladocora caespitosa*, *Oculina patagonica* and *Eunicella singularis* and of the asymbiotic *Corallium rubrum* (Rodolfo-Metalpa et al., 2005, 2006a, 2006b; Torrents et al., 2008; Ferrier-Pages et al., 2009). The response of these species to temperature has been mainly examined at a local scale (i.e. one or two populations, Rodolfo-Metalpa et al., 2006, Torrents et al., 2008; Ferrier-Pages et al., 2009). But, thermal thresholds vary by region (Wellington et al., 2001; Riegl, 2003; Oliver et al., 2009) which would be consistent with the observed large variability in the effects of mass mortality events (Perez et al., 2000; Cerrano et al., 2000; Garrabou et al., 2009). Then, thermotolerance studies that include populations from different

spatial scales are necessary to contribute the understanding of the causes and consequences of these events and their impact variability.

Among the main biological factors and mechanisms determining differential responses, acclimatization (i.e. the modification in physiological traits in response to changes in environmental variables; Wilson and Franklin 2002) and/or local adaptation (i.e. the patterns and processes driven by divergent selection, leading the locally adapted genotypes to have on average higher relative fitness in their habitat than foreign genotypes; Williams 1966; Kawecki and Ebert 2004) could play a major role (Williams, 1996; Wilson and Franklin, 2002, Kawecki, 2004; Ledoux, 2010). For the Mediterranean species, a recent study based on field experiments with red coral *C. rubrum* populations dwelling in contrasted temperature regimes suggested the existence of differential acclimatization capacities and the action of local adaptation in the studied populations (Ledoux, 2010). Moreover, previous experimental studies also found differences in the response to temperature between populations dwelling in different temperature regimes (Torrents et al. 2008; Ferrier-Pages et al. 2009). However, although these processes could play a major role in modulating the response of populations to ongoing warming, knowledge about their occurrence and relevance is still scarce for temperate marine species.

In this study we carried out a comparative thermotolerance study with populations of the red gorgonian *Paramuricea clavata* from the NWM region dwelling in distinct temperature regimes and which displayed differential mortality rates during the last MME events at different spatial scales (i.e. local to regional, Perez et al., 2000; Cerrano et al, 2000; Garrabou et al., 2009). The aim of this experimental study was to provide basic information on thermotolerance features of *P. clavata* and to determine possible thermotolerance differences of populations dwelling in contrasted temperature regimes with the purpose to assess the potential warming associated risks for the conservation of the populations.

2. Materials and Methods

2.1. Study area

The study was carried out with populations dwelling in three areas of the NW Mediterranean basin that displayed differential temperature regimes (Bensoussan et al. 2010). From west to east these areas were: Illes Medes (L'Estartit, NE Spain), Marseille (France), and Scandola (NW Corsica, France) (Fig. 1).

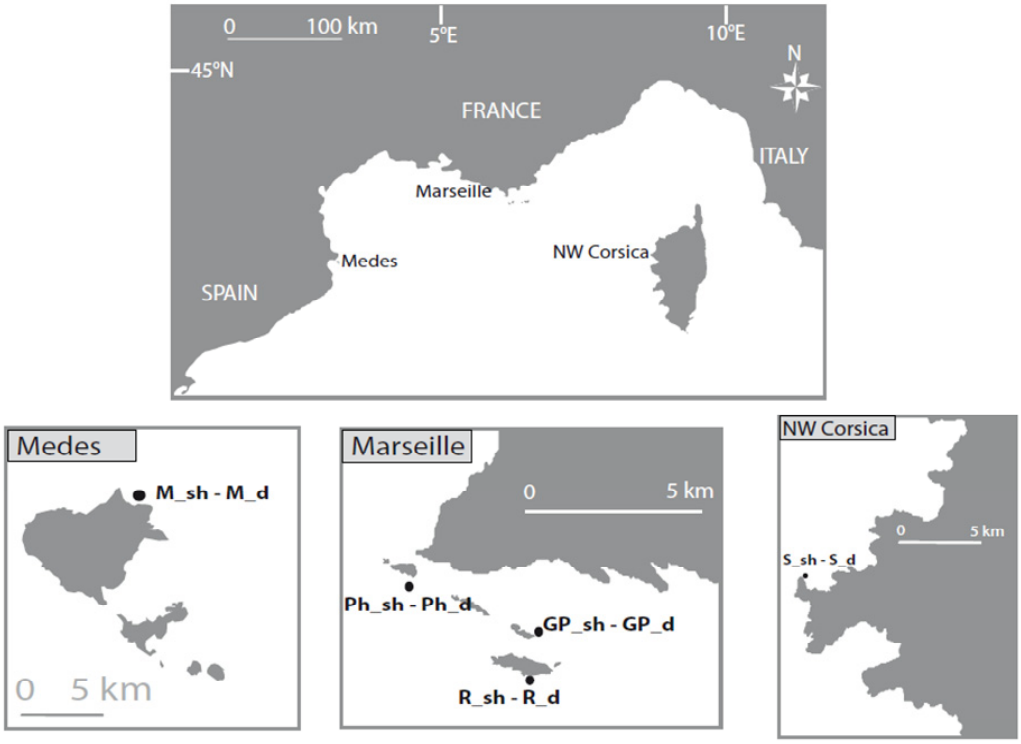

Figure 1. Northwestern Mediterranean region and detail of study sites with locations where colonies were sampled.

2.2. Model species

The red gorgonian *P. clavata* was chosen as model species to perform the experiments. This species is considered one of the key species of Mediterranean coralligenous assemblages (True, 1970; Gili and Ballesteros, 1991). Moreover, this species was one of the most affected in the recent mass mortality events (MME) occurred in the NW Mediterranean coastal waters (Cerrano et al., 2000; Perez et al., 2000; Linares et al. 2005; Garrabou et al., 2009).

2.3. Experimental setting and biological material

Experiments were carried out with apical colony's tips (between 5 and 7 cm in length) of healthy red gorgonian colonies randomly sampled using SCUBA diving from each population. Each colony's apical tip (hereafter colony) was assembled in experimental plates. These plates were built up by three PVC rectangular pieces (5 x 30 cm in size) and two rubber layers. Each PVC piece had 10 to 12 holes (1 cm in diameter) to place the colonies (Fig. 2). The PVC plates and rubber layers were hold together with plastic screws. The colonies were fixed in the experimental plates without using any putty, instead the colonies were hold mechanically thanks to the perpendicular cuts previously done in the rubber layers (one cut in each layer) at the holes level (Fig. 2).

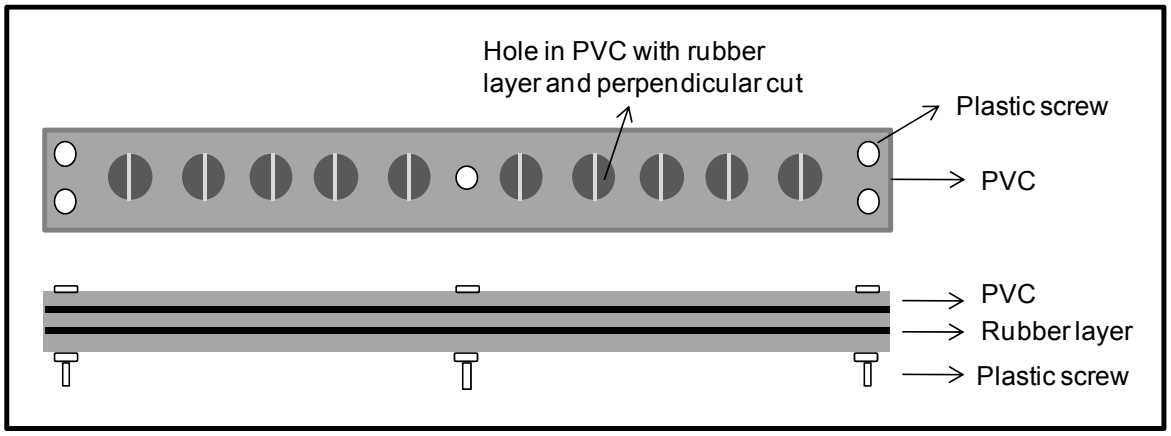

Figure 2. Outline (upper and side view) of PVC plates where colonies were placed. Model plates used in the aquariums of the *Institut de Ciences del Mar* are presented; *in the Station Marine d'Endoume* same general design was used but holes in PVC were placed differently.

Once the colonies were placed in the experimental plates were either set directly in the experimental tanks or maintained in an aerated cooled water box for transportation. The experiments were carried in the experimental aquariums facilities of the *Station Marine d'Endoume* in Marseille (France) and those of the *Institut de Ciences del Mar* in Barcelona (Spain) (Fig. 3a and 3b). The maximum transportation time was of 36h for colonies collected in Scandola (Corsica, France) and transferred to the Barcelona's experimental aquarium facilities.

All experiments used the same experimental setting with two main aquarium sets: Control and T Treatment (Fig. 4). Both Control and Treatments sets were composed of three tanks (≈ 15 l in Marseille, ≈ 105 l in Barcelona) where colonies were placed plus one large buffer tank (≈ 75 l in Marseille, ≈ 100 l in Barcelona) (Fig. 4). The buffer tanks were supplied with Mediterranean seawater (pumped at 3 m depth in Marseille and at 15 m depth in Barcelona)

and from there pumped continuously into the experimental tanks. Each tank had an inlet pipe for the supply of fresh sea water, and an outlet pipe. Both pipes remained open so that the tanks functioned as an open system.

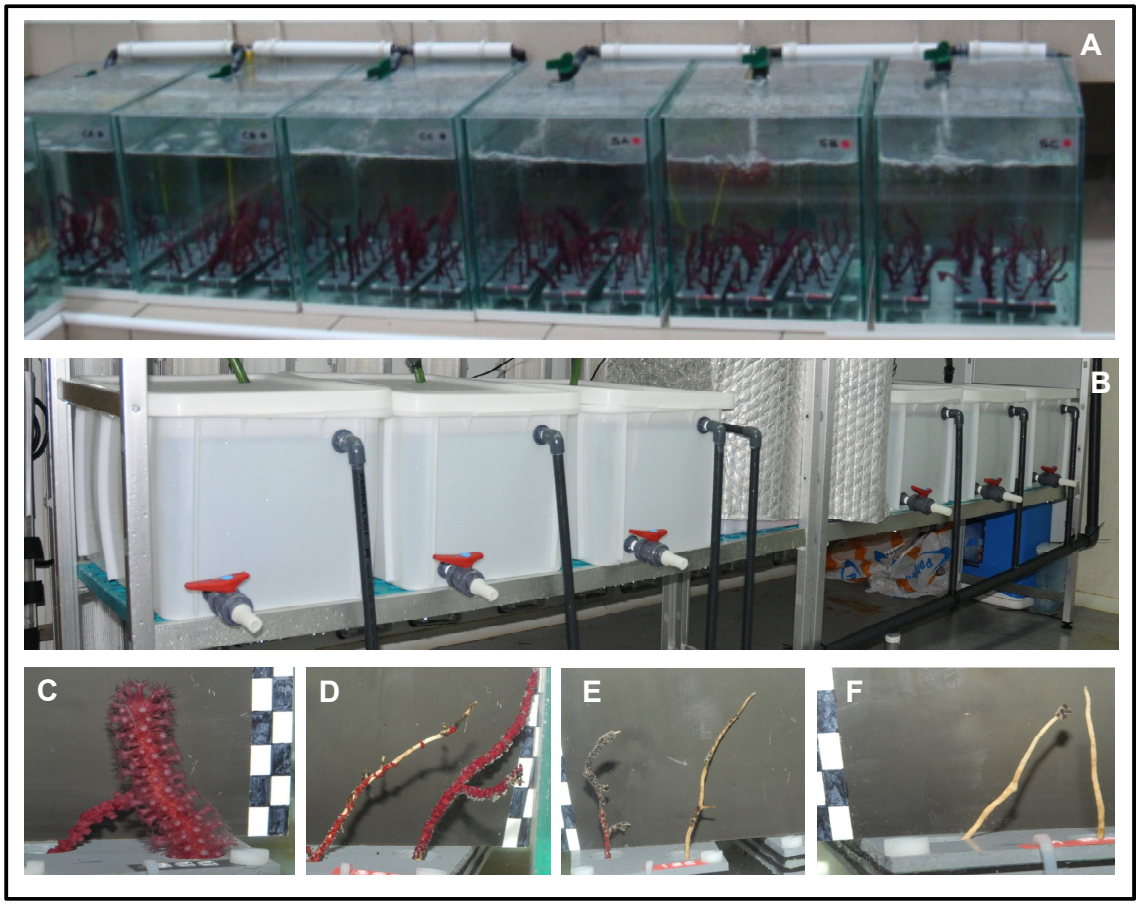

Figure 3. Aquariums of the *Station Marine d'Endoume* containing colonies in PVC plates (A), aquariums of the *Institut de Ciences del Mar* (B) and colonies with different degree of affectation, from 0 to 100 % of tissue necrosis (C, D, E and F).

In the Treatment set, seawater was heated in the buffer tank with submersible resistance heaters and was regulated by temperature controllers (Aqua Medic T controller). Temperature was registered in Control and Treatment aquariums with Tidbit Stowaway autonomous temperature sensors every half an hour. Finally, experimental tanks were equipped with submersible pumps to facilitate water circulation within the tanks.

2.4. Colonies feeding

Colonies were fed 2 days per week, one day with nauplii of *Artemia salina* and the other with phytoplankton diatom *Tetraselmis chuii* (for information of food concentrations see Annex 2). After the addition of phytoplankton and zooplankton, the tanks were maintained in closed system mode for 5 h (during this period water circulation within tanks was supplied by submersible pumps). Previous experimental studies with *P. clavata* indicated that 5 h is an appropriate period, after which food abundance reach natural levels (Coma et al., 2009). The strategy of supplying pulses of food instead of a constant supply was based on the results of *in situ* studies of food abundance and prey capture at short time scales (Rossi, 2002; Coma et al., 2009). The results of sampling food abundance near the organisms and their gut contents demonstrated that organisms commonly acquire available resources in short time pulses. There was estimated that these pulses of food abundance occurred between 2 and 3 times per week on average (Rossi, 2002; Coma et al., 2009), so that was simulated in performed experiments. For more details in food supply see Coma et al., (2009).

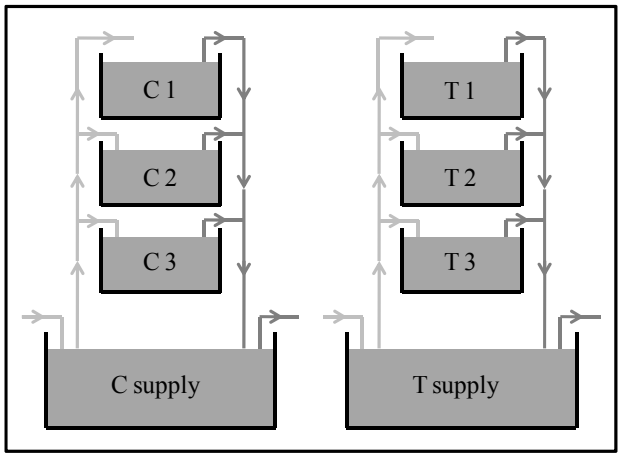

Figure 4. Basic experimental design of all performed experiments. C1, C2, and C3: Control aquariums; T1, T2 and T3: Treatments aquariums. C and T supply: buffer tanks of Controls and Treatments respectively. Arrows indicate water flow.

2.5. Experimental designs

We run a total of 7 thermotolerance experiments concerning the sampling of almost 1000 apical parts of colonies from different populations (Table 1). All experiments were carried out between March and November of 2009. Control tanks were maintained at 16 to 17ºC, while in T Treatment tanks different temperatures (ranging from 22 to 28ºC) were applied. The experiments were organized in three blocks to assess: 1) Differential response of

populations dwelling in contrasted temperature regimes, 2) Upper thermal limit of populations, and 3) Seasonal changes in thermotolerance response (Table 1).

Table 1. Principal characteristics of preliminary experiments and of three experimental blocks performed in this work. Num. col. (C/T): number of colonies per population and per aquarium at Controls and Treatments. R_sh: Riou shallow, Ph_sh: Pharillons shallow, GP_sh: Grotte Pérès shallow, M_sh: Medes shallow, M_d: Medes deep, S_sh: Scandola shallow, S_d: Scandola deep.

	T (°C)	Sample date	Stabulation. (days)	Exp. dates	Dur. (days)	Num. col. (C/T)	Population
Preliminary	18-27	11 March	8	19/03 - 14/05	57	10/10	R_sh, Ph, sh, P_sh
Inter-region, inter-depth	18-25	18-19 June	13-14	1/07-1/09	63	10/10	M_sh, M_d, R_sh, Ph_sh, P_sh, R_d, S_sh, S_d
Upper thermal limit	26±0.5	18-19 June	77-78	3/09-14/09	12	5/5	M_sh, M_d, R_sh, Ph_sh, P_sh, R_d, S_sh, S_d
	27±1	18-19 June	139-140	5/11-9/11	5	*/5	
	28±0.8	25 October	14	8/11-11/11	4	*/10	M_sh, M_d
Season effect	25±0.1	11 March	72	22/05-14/06	24	7/3	R_sh, Ph, sh, P_sh
	25±0.1	18-19 June	45-46	3/08-26/08	24	10/10	
	25±0.7	3 September	13	16/09-9/10	24	5/10	

*No controls were available

2.5.1. Preliminary experiment: testing for critical temperatures and intra-regional variability

A preliminary experiment was carried out to asses (1) the critical T for *P. clavata* populations and (2) to analyze the variability among populations dwelling under the same temperature regime (intra-regional scale). This experiment was intended to provide guidelines on the critical temperature values for further T treatments and establish the number of populations to be used per region and depth range in the thermotolerance experiments. With this last objective we compared coenenchyme necrosis along the experiment of the three populations.

The experiment started the 11th March 2009. Three populations dwelling at 20 m depth from the Marseille areas were sampled (Grotte Pérès, Pharillons, Riou). From each population 60 colonies (30 for the Control and 30 for T treatment) were used resulting in a total of 180 colonies. Colonies were exposed to a *leader* T regime of gradual T augmentation from 18 to 27°C. Temperatures of 18, 20, 22, 23, 24, 25 and 26°C were consecutively maintained for one week, and finally colonies were exposed 9 days at 27°C (Table 1). At the last

experiment day 100% of colonies presented some level of coenenchyme necrosis (see section 2.6).

2.5.2. Response of populations dwelling in contrasted temperature regimes

To compare the response to T of populations dwelling in contrasted temperature regimes, we used populations dwelling in three NW Mediterranean areas (Medes, Marseille and Scandola) and within each region we considered populations dwelling in two depth ranges corresponding to the thermocline and subthermoclinal levels (20 and 40 meters respectively, Table 1). In a previous study, summer temperature regimes from these regions and depths ranges were characterized and highlighted very distinct temperature conditions among them (for more information see Bensoussan et al., 2010 and Annex 2). In summary, at 20 m depth, Marseille exhibited the coldest summer conditions (Mean summer T ≈ 18^{o}C), while Scandola was the warmest site (Mean summer T ≈ 21^{o}C, but reaching Maximum Ts of ≈ 27^{o}C), being Medes in an intermediate position (Mean summer T ≈ 20^{o}C). At 40 m depth Marseille and Scandola were the coldest regions (Mean summer T ≈ 16^{o}C) and Medes the warmest one (Mean summer T ≈ 17^{o}C, but reaching Maximum Ts of ≈ 25^{o}C, 1.5 to 2^{o}C higher than the other areas) (Annex 2). With respect to the variability of the summer thermal regime, clear inter-regional differences were also observed. Marseille was the most variable site at 20 m (Coefficient of variation ≈ 18%) because of the occurrence of upwellings, while Medes exhibited the highest variability at 40 m (Coefficient of variation ≈ 17%) as cause of the recurrent downwellings (Bensoussan et al., 2010, Annex 2).

For this experiment we used a total of 8 populations, two from Medes Islands (Pota del Llop 20 and 40 m), two from Scandola (Palazzinu 20 and 40 m) and 4 from Marseille (Grotte Pérès and Pharillons 20 m and Riou 20 and 40 m) (Fig. 1, Table 1). The preliminary experiment did not display significant differences on response to T stress among populations dwelling at the same depth (see Results section). Based on this we decided to consider only one population per depth range in each region. However, in the Marseille region we maintained three populations from 20 m in order to evaluate potential differences between populations dwelling under the same temperature regime and collected in the late spring. Unfortunately, we could not extent this study to other depths or regions due to experimental constrains.

A total of 480 colonies (60 per population) were collected by scuba divers in 18-19 June 2009 and transported to the Barcelona experimental aquarium facilities in less than 36 hours after sampling (see section 2.3). Colonies collected from each population were placed in 6

experimental plates (10 colonies per plate), which then were distributed in the Control and T Treatments tanks (one plate in each tank). For this experiment, populations were exposed consecutively to 18, 20, 22, 23 and 24°C (one week each T) and finally for 30 days to 25°C (Table 1). We retained 25ºC as final treatment T since at this T, populations started to show signs of necrosis in the preliminary experiments (see Results) while at higher T, colonies were severely affected in few days (see Results). Therefore we considered that 25ºC was an optimal T to explore potential differences in thermotolerance between populations. The last day of the experiment, 97% of the colonies presented some degree of coenenchyme necrosis.

2.5.3. Assessing P. clavata upper thermal limits

To know the response to T above 25ºC of *P. clavata* populations we performed experiments with constant Ts at 26, 27 and 28°C. The 26 and 27°C experiments were carried out with the same populations used in the previous experiment. For each one, half of colonies of controls of the inter-regional and inter-depth experiment were used (Table 1). First, we performed the 26°C experiment (started at the beginning of September 2009) and after the 27°C (started at the beginning of November 2009) (Table 1). Finally, for the 28°C experiment we only considered populations from Medes region (Pota de Llop 20 and 40m) collected in October 2009 (experiment started in the begging of November; Table 1). As in other experiments we sampled 60 colonies per population which were placed in experimental plates and distributed in the experimental tanks as described previously (Table 1). For the three experiments, the last day of the experiment, 98 – 100% of the colonies presented some degree of necrosis.

2.5.4. Seasonal effect in P. clavata response to temperature

From previous studies we do know that physiological status varies seasonally (Coma et al., 2000). In particular during spring *P. clavata* populations devote most of their energy to gonadal development (Coma et al. 1995a) and at the end of spring early summer spawning of gonads occur (Coma et al. 1995b). Moreover, it was found that the summer period, characterized by warm T and reduced food resources, leads to physiological stress (Coma and Ribes, 2003; Coma et al. 2009). We wanted to test how the changes in the physiological status could affect the response of populations to T stress.

In this experiment, we used colonies of three shallow populations (20 m) from the Marseille region (Riou, Pharillons and Grotte Pérès) collected at three different times during the year:

late winter (11 March 2009), late spring (18 June 2009) and late summer (3 September 2009) (Table 1). For each different time the colonies were exposed to 25°C for 24 days.

For the late-winter experiment part of colonies from the Control set used to perform the preliminary experiment were used (see above). These colonies were stabulated 61 days under control conditions before the experimentation (Table 1). For the late spring experiment, the results from inter-regional and inter-depth experiments were used. More precisely we considered the data set corresponding to the 24 first days of exposure to 25°C. In this case, these colonies were submitted to 46 stabulation days (including 13 stabulation days in control conditions and 33 days of augmentation form 18 to 24°C). Finally, the late summer experiment colonies were maintained during 13 days under control conditions until they were submitted to 25°C (Table 1). In the last day of these experiments, many colonies were not affected with necrosis since the length of the late-spring and the late-summer experiments were equaled to late-summer experiment (shortest experiment). This experiment was finished before most of the colonies were affected with coenenchyme necrosis because of experimental constraints.

2.6. Response variable

The measured response variable was the coenenchyme necrosis, it was monitored daily by visual inspection and was assessed as the proportion of the colony with dead tissue (from clear grey to black) or denuded axis (Fig. 3c, d, e and f).

2.7. Statistical methods

To compare pattern in tissue necrosis among populations of the same region, among regions/populations (same depth) and depths (all regions together and inside regions) and among seasons we performed univariate PERMANOVA analyses (Anderson, 2001; McArdle and Anderson, 2001) in PRIMER v6.1.11 (Clarke and Warwick, 2001). We chose this non-parametric method because nor normality or homocedasticity were found in the dependent variable. (Anderson, 2001; McArdle and Anderson, 2001). This method uses Euclidean distance as the basis of the analysis including time as an additional fixed factor (see Anderson et al., 2008 for full methodology) and relies on comparing the observed value of a test statistic (pseudo *F*-ratio) against a recalculated test statistic generated from random re-ordering (permutation) of the data (Anderson, 2001). For all test, 9999 permutations were performed. To simplify the analyses implementation, for the factor time (experiment day) tissue necrosis data from three to four days covering the whole experiment duration were

chosen to run the analyses. The first day corresponded with the day where the first signs of necrosis occurred and the third/fourth day was the last experiment day. In the case where only three days were considered, the second day was the one in the middle of the first and the third day. In the case where four days were considered, the second and third days where the ≈0.25 and ≈0.75 quantiles of the variable time (considering the period between the day with the firsts signs of necrosis and the last experiment day). Before running analyses to respond proposed questions, comparisons between replicates (aquariums) were made. From a total of 105 pair-wise comparisons of all population among replicates through all performed experiments only a 5% presented statistical differences in populations responses between aquariums (the statistical differences were found within high T experiments where it was more difficult to maintain exactly the same T among aquariums as cause of heat loss) so to simplify tests designs we considered data from three replicates together. No tests were performed to control experiments since at all experimental sets, control colonies remained healthy (no tissue necrosis) until the end of the experiments.

Test designs

For the preliminary experiment the Factors Population and Time (experiment day) were considered to compare the coenenchyme necrosis. The Factor Population was set as random since we intended to attest for the variability in the response to T among populations of the same region under the null hypothesis that no inter-population differences will arise. On the contrary the Factor Time was set as fixed as we chose different experiment days with the intention to capture low to high levels of coenenchyme necrosis (Table 2a).

For the inter-regional/inter-depth experiment, the Factors Region, Depth and Time were considered. Inside Marseille region we kept only populations from Riou since it was the Marseille' site form which we had populations of both depths. For this design all Factors were fixed. Different regions and depths were chosen because they display contrasted thermal regimes (see section 2.5.2 and Annex 2) and as mentioned above different experimental days (Time) were selected to capture from low to high coenenchyme necrosis levels (Table 2b).

Finally, to asses for intra-regional differences with June collected populations, we compared the coenenchyme necrosis of Marseille shallow populations. The same design of that of Preliminary experiment was carried out (Table 2c).

For each of the upper thermal limit experiments (26, 27 and 28^{o}C), all populations were considered together in the same Factor (Population) which was set as random. As before, factor Time was set as fixed (Table 4).

To evaluate the effect of the season in the three shallow Marseille populations in the response to T we considered the Factors Season and Population. The Season was set as fixed since different seasons were chosen under the hypothesis that populations present different physiological state within them, while the factor Population was set as random. (Table 7).

In general, when any of the performed analyses indicated significant differences between populations/regions/depths/seasons, pair-wise test were performed to know between which pairs of the factors levels the differences occurred.

2.8. Assessing mass mortality risks and consequences on P. clavata populations

In this section, firstly we compiled the response of *P. clavata* to temperature from experimental data and compared to the T conditions measured in the field. And secondly we analyzed number of resistant colonies within populations. The intention of these analyses was to assess for the risk of mortality of *P. clavata* populations in the different regions and depth ranges and to evaluate the capability of populations in facing future T anomalies.

Thermotolerance curve versus field temperature conditions. A thermotolerance curve was fitted to the experimental data to illustrate T conditions that lead to *P. clavata* mortality. The day related to the first signs of affectation (we considered that a colony was affected when it reached 10% of tissue necrosis or denuded axis) of each population from all performed experiments except those testing the seasonal effect were considered to construct the thermotolerance curve. Besides we also add data from the previous experimental works on *P. clavata* thermotolerance (Coma et al. 2009). This curve was compared with field T conditions found in the three study regions considered here. To characterize T conditions we selected the number of days of the summer period above different Ts from 23 to 28 ºC. To obtain these values we considered inside each region and T the year with higher duration above the considered T. The available period of T series in Medes, Marseille and Scandola was 2002-2010, 1999-2010 and 2004-2010 respectively (Annex 2). Since we disposed of hourly T records, to calculate the number of days above each considered T, we counted the number of hours above a specific T during the summer period (1 July to 30 September as defined in Chapter 2), and then we transformed the number of hours in days (so presented number of days does not correspond with natural days). For each region, analyzed shallow

field T data was that of the depth that correspond with *P. clavata* upper depth limit, which in Medes and Marseille correspond to ≈ 10 m and in Scandola to ≈ 20 m, while for all regions, analyzed deep T conditions were those of 40m depth.

Percentage of resistant colonies. We analyzed inside each studied population the number of resistant colonies at intermediate experiment day (the second or third day of the factor time chosen to perform PERMANOVA) of different experiments to evaluate the resistance to different Ts (25 to 28ºC). The election between the second and third day was done considering the number of days that was nearer to the most extreme conditions observed in the field, although, in general, the number of selected days was higher than those ever registered *in situ.* Previous field studies of populations affected by mass mortality events indicated that colonies with low levels of injury (< 33% colony necrosis) had higher chance to survive several years after the event (Linares et al., 2005) and that they maintained, one year after the event, similar proportion of fertile polyps than non affected colonies (Linares et al., 2008) so we considered as resistant a colony which presented less than 30%.

3. Results

3.1. Preliminary experiments: testing for critical temperatures and intra-regional responses

In this experiment the three shallow populations from Marseille' region showed the first signs of necrosis at 25°C (Fig. 5). While at 26°C and 27°C, tissue necrosis augmented very rapidly, presenting all populations after one week at 26°C ≈ 40% of coenenchyme necrosis in average, and after 9 days at 27°C values of more than 90% (Fig. 5). The three populations presented similar values of coenenchyme necrosis with the increasing T treatment and accordingly, PERMANOVA tests did not detect statistical differences among populations (Fig. 5, PERMANOVA p-value >0.05, Table 2a).

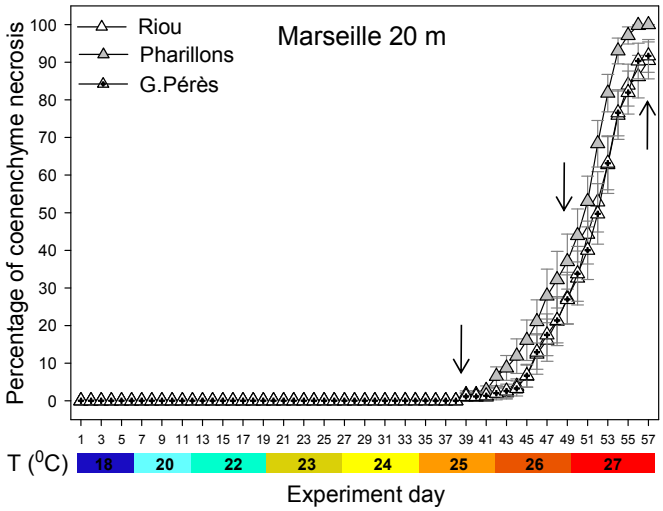

Figure 5. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from shallow (20 m) populations of Marseille submitted to a leader T regime (18 to 27°C). Colonies were collected in March. Arrows indicate selected days to perform PERMANOVA analyses.

Based on the results of this preliminary experiment we decided to retain 25ºC as T treatment for comparative thermotolerance experiments since at this T colonies started to show the firsts signs of tissue necrosis (Fig. 5). Likewise, the lack of differences in the response of the three populations dwelling under the similar temperature regime seemed to support the use of one population per region and depth range to infer differential inter-regional and inter-depth responses (but see below).

Table 2. PERMANOVA designs and results for the coenenchyme necrosis comparisons of the preliminary (a) and the inter-region/inter-depth experiments (b). Intra-regional comparisons were carried out with Marseille shallow populations (c). Each section of the table corresponds to one PERMANOVA analysis. The Population/Region refers to the selected populations in a) and c) and regions in b) (in parenthesis we indicated the population selected). The Time refers to the experiment days selected to perform PERMANOVA analyses. ***: p-value<0.001, ** : p-value<0.01, *: p-value<0.05, ns: not significant.

		Factor	Factor type.	Depth	Population/Region	Time	Necrosis (p-value)
a	**Preliminary experiment**	Site	Random	20 m	Riou, Pharillons, Grotte Pérès	39, 49, 57	ns
		Time	Fixed				***
		Pop. x Time	-				ns
b	**Inter-regional /inter-depth**	Region	Fixed	20 and 40 m	Marseille (Riou), Medes, Scandola	34, 44, 54, 63	***
		Depth	Fixed				**
		Time	Fixed				***
		Region x Depth	-				ns
		Region x Time	-				**
		Depth x Time	-				ns
		Re x De x Ti	-				ns
c	**Intra-regional Marseille shallow pops.**	Site	Random	20 m	Riou, Pharillons, Grotte Pérès	34, 44, 54, 63	**
		Time	Fixed				**
		Pop. x Ti	-				**

3.2. Inter-regional and inter-depth comparisons

When regarding shallow populations (20 m), the population from Scandola was the first in displaying necrosis and the one that showed the highest necrosis rates all along the experiment (Fig. 6a). Medes and two populations from Marseille' region (Riou and Pharillons) showed intermediate values of necrosis (Fig. 6a). Finally, Marseille population Grotte Pérès displayed the lowest percentage of tissue necrosis of all populations all along the experiment (Fig. 6a). As an example of the contrasted response to T stress, Scandola displayed at the 54 and 63 experiment days an average of ≈ 43 and ≈ 78% of coenenchyme necrosis respectively, while for the same days in Grotte Pérès these values corresponded with ≈ 7 and ≈ 40% (Fig. 6a).

Regarding deep populations, again Scandola was the most affected region, followed by Medes and finally by Riou (Fig. 6b). In the last experimental day, Scandola presented in average ≈ 91%, Medes ≈ 73% and Riou ≈ 62% of coenenchyme necrosis (Fig 6b).

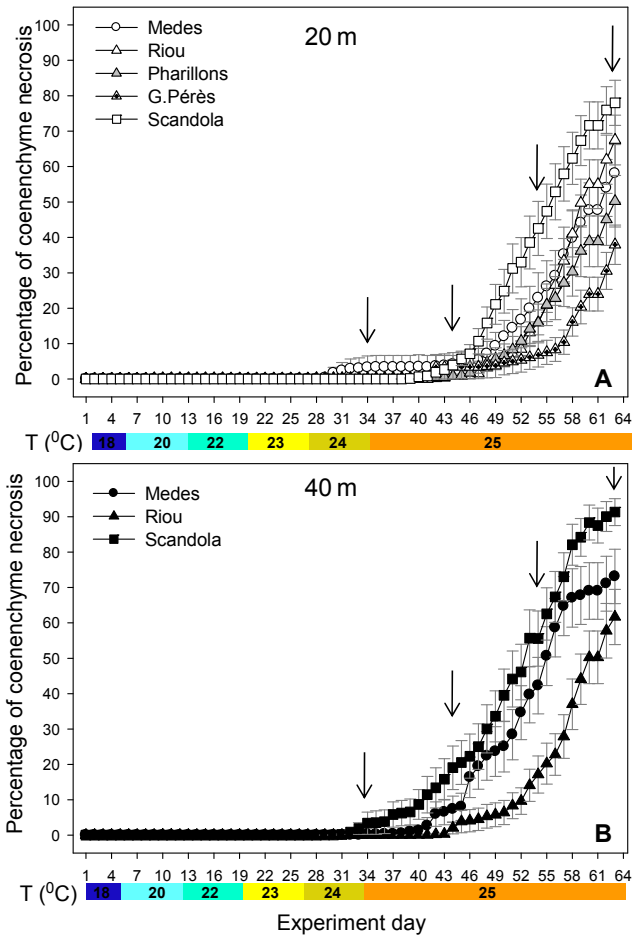

Figure 6. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from shallow (20 m, A) and deep (40 m, B) populations of Medes, Marseille and Scandola submitted to a leader T regime (18 to 25°C). For the Marseille region at 20 m depth three populations were considered (Riou, Pharillons and Grotte Pérès) while for 40 m depth only Riou population was available. Colonies were collected in June. Arrows indicate selected days to perform PERMANOVA analyses.

When analyzing inter-depth differences, considering all populations together within each depth (but without considering Pharillos and Grotte Pérès in Marseille' region), deep populations (40 m) presented higher percentage of coenenchyme necrosis than shallow populations (20 m), although, differences were not very important (Fig. 7a). With the aim to represent inter-depth differences of each region separately, we performed a bar graph with coenenchyme necrosis of the last experiment day (Fig. 7b). When analyzing inter-depth differences within Marseille (Riou), shallow populations accounted for higher coenenchyme necrosis than deep populations. At the end of the experiment, shallow populations presented ≈ 67% while deep populations ≈ 62% of mean coenenchyme necrosis (Fig. 7b). At Medes and Scandola, shallow populations presented lower levels of necrosis than deep populations. For Medes, at the end of the experiment, shallow populations presented ≈ 58%

while deep populations presented ≈ 70% of coenenchyme necrosis in average. At Scandola, last experiment day presented ≈78% of mean coenenchyme necrosis in shallow populations, while ≈91% at deep populations (Fig. 7b).

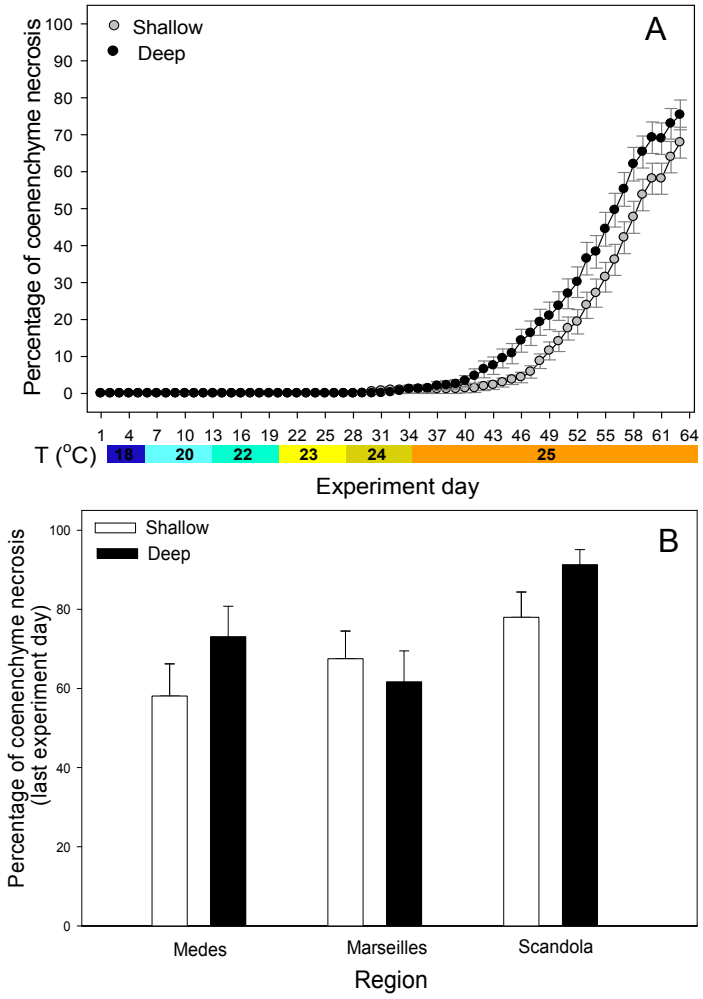

Figure 7. Mean percentage of coenenchyme necrosis (±SE) of *P. clavata* colonies of the inter-regional/inter-depth experiment (18 to 25°C) of shallow (20 m) and deep (40 m) populations considering all regions together (A) and each region separately (B). In B) bars represent last experiment day coenenchyme necrosis (Mean ±SE). For the Marseille region only Riou was considered since it was the only site were both depths were sampled.

PERMANOVA inter-regional/inter-depth analysis accounted for significant differences between regions, depths and time (Table 2b). Pair-wise comparisons between regions and within depths, indicated for the shallow populations (20 m), significant differences between Scandola and Marseille, and between Scandola and Medes. No differences were found between Medes and Marseille (Table 3). Regarding deep populations (40m), each pair-wise comparison was statistically significant, being the greatest differences observed between Scandola and Riou (Table 3). Pair-wise comparison between depths (within regions)

accounted for a lack of differences between Marseille shallow and deep populations (considering only Riou populations) (Table 4). At Medes and Scandola higher affectation of deep populations was found to be significant (Table 4)

Intra-regional comparisons of shallow Marseille populations indicated significant differences between populations and among time (Table 2c). Pair-wise comparisons between this populations indicated significant differences between Riou and Grotte Pérès (Table 5).

Table 3. PERMANOVA pair-wise comparisons within shallow (upper-right) and deep (lower-left) populations of the three considered regions in the inter-regional/inter-depth experiment. Inside Marseille region only Riou populations were considered for this analysis. ***: p-value<0.001, **: p-value<0.01, *: p-value<0.05, ns: not significant.

Deep\Shallow	Medes	Marseille	Scandola
Medes		ns	*
Marseille	*		**
Scandola	*	***	

Table 4. PERMANOVA comparisons between shallow and deep population within the three study regions considered in the inter-regional/inter-depth experiment (for Marseille region only Riou populations were considered). ***: p-value<0.001, ** : p-value<0.01, *: p-value<0.05, ns: not significant.

Region	**p – value**
Medes_sh – Medes_d	*
Marseille_sh – Marseille_d	ns
Scandola_sh – Scandola_d	**

Table 5. PERMANOVA pair-wise comparisons within shallow Marseille populations of the inter-regional/inter-depth experiment. ***: p-value<0.001, ** : p-value<0.01, *: p-value<0.05, ns: not significant.

Population	**p – value**
Riou – Phar	ns
Riou – G. Pérès	**
Phar – G. Pérès	ns

3.3. Assessing *P. clavata* upper thermal limit

When exposed to 26, 27 and 28°C, all populations attained high levels of tissue necrosis after just few days (Fig. 8). At 27 and 28°C experiments in less than 5 and 4 days all colonies were dead (Fig. 8b and 8c). In the 26°C experiment colonies suffered a more delayed response to T and some inter-population differences could be observed (Fig. 8a). Medes shallow and deep populations showed a rapid increase of necrosis, reaching in the

6th day in average more than 80% of coenenchyme necrosis, and at the end of the experiment ≈ 100% (Fig. 8a). The remaining populations presented instead a more linear response until the last day of the experiment, being Grotte Pérès and Riou shallow populations the least affected, presenting although high levels of coenenchyme necrosis at the end of the experiment (12 days) with values ranging between 60 and 75% of coenenchyme necrosis, respectively (Fig. 8a). For the 26°C experiment, more than half of the pair-wise comparisons presented significant differences, involving mainly Medes shallow and deep populations but also Grotte Pérès shallow and Riou shallow (Table 6a and 7).

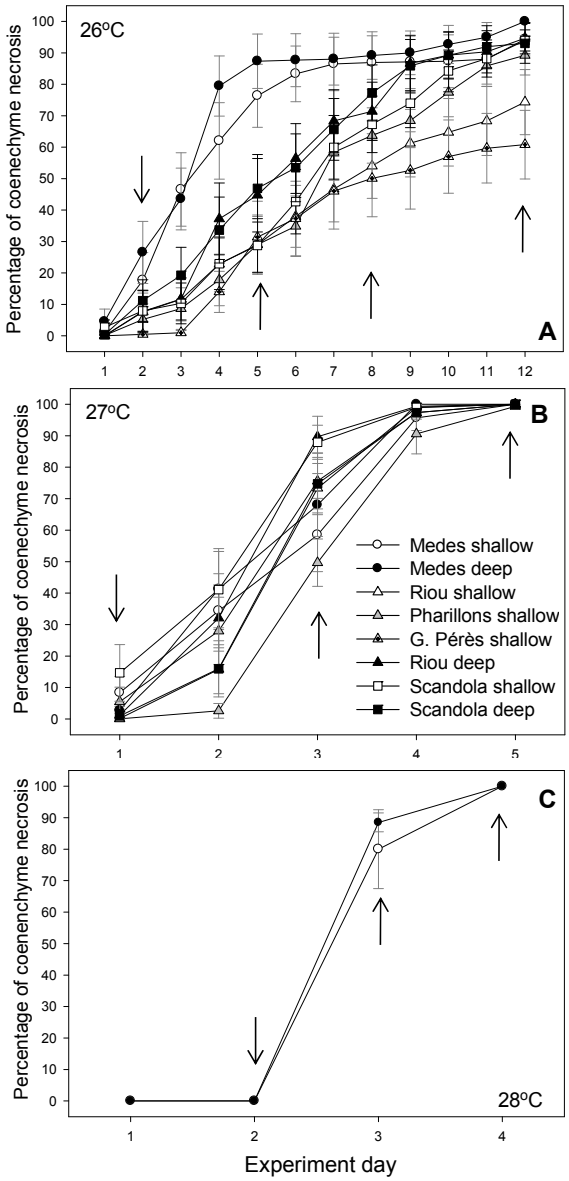

Figure 8. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from populations of Medes, Marseille and Scandola submitted to 26 and 27°C and from populations of Medes submitted to 28°C. Colonies for the 26 and 27°C experiments were collected in June, while those for the 28°C experiment were collected in October. Arrows indicate selected days to perform PERMANOVA analyses.

In the 27°C experiment there were significant differences between populations (Table 6b) involving mainly Scandola shallow but also Grotte Pérès shallow, Riou deep and Scandola deep populations (Table 7). On the contrary, in the 28°C experiment no significant differences were found between populations (Table 6c)

Table 6. PERMANOVA designs and results for the coenenchyme necrosis comparisons of the 26°C, 27°C and 28°C experiments. The Factor Population refers to the selected populations to perform the comparisons, the Time refers to the experiment days selected to perform PERMANOVA analyses R_sh: Riou shallow, Ph_sh: Pharillons shallow, GP_sh: Grotte Pérès shallow, M_sh: Medes shallow, M_d: Medes deep, S_sh: Scandola shallow, S_d: Scandola deep. ***: p-value<0.001, **: p-value<0.01, *: p-value<0.05, ns: not significant.

		Factor	Factor type	Population	Time	Necrosis (p-value)
a	**26°C**	Population	Random	M_sh, M_d, R_sh,	2, 5, 8, 12	***
		Time	Fixed	Ph_sh, GP_sh,		***
		Pop. x Time	-	R_d, S_sh, S_d		***
b	**27°C**	Population	Random	M_sh, M_d, R_sh,	1, 3, 5	**
		Time	Fixed	Ph_sh, GP_sh, R_d		***
		Pop. x Time	-	S_sh, S_d		*
c	**28°C**	Population	Random		2, 3, 4	ns
		Time	Fixed	M_sh, M_d		**
		Pop. x Time	-			ns

Table 7. PERMANOVA pair-wise comparisons between populations of the 26°C (upper-right) and 27°C (lower-left) experiments. ***: p-value<0.001, ** : p-value<0.01, *: p-value<0.05, ns: not significant.

27°C\26°C	M_sh	M_d	R_sh	Ph_s	GP_sh	R_d	S_sh	S_d
M_sh		ns	***	***	***	*	**	*
M_d	Ns		***	***	***	***	***	**
R_sh	ns	ns		Ns	ns	*	ns	*
Ph_sh	ns	ns	ns		ns	ns	ns	ns
GP_sh	ns	ns	*	*		**	*	**
R_d	ns	ns	ns	Ns	***		ns	ns
S_sh	*	*	*	Ns	***	ns		ns
S_d	ns	ns	ns	Ns	*	ns	ns	

3.4. Seasonal effect in *P. clavata* response to temperature

No significant differences were found in the percentage of coenenchyme necrosis when comparing shallow Marseille populations collected in different seasons, instead significant differences were found between populations (Table 8).

Table 8. PERMANOVA designs and results for the coenenchyme necrosis comparisons of the inter-season experiment (25°C). Inter-site comparisons were also performed. ***: p-value<0.001, **: p-value<0.01, *: p-value<0.05, ns: not significant.

Factor	Factor type	Season	Population	Time	Necrosis (p-value)
Season	Fixed				ns
Population	Random				*
Time	Fixed				**
Season x Pop.	-	March, June, September	Riou, Pharillons, G. Pérès	11,16 , 20, 24	ns
Season x Time	-				ns
Pop. x Time	-				ns
SexPop.xTi	-				ns

Since no differences were observed between seasons (but yes between populations), we were interested in knowing potential differences between populations inside each season.

In March, all populations presented in the last experiment day ≈ 25% of tissue necrosis in average (Fig. 9a) while in June, important differences between Riou/Pharillons (≈ 33/27%) and Grotte Pérès (≈ 10%, Fig. 9b) were observed. For September collected populations these differences were also important presenting Riou and Pharillons during the last experiment day an average of ≈ 20% and Grotte Pérès less than 6% of coenenchyme necrosis (Fig. 9c).

PERMANOVA analysis considering populations inside each season indicated no significant differences between March populations. In June and September, Grotte Pérès (which showed to be the most resistant population) presented significant differences in coenenchyme necrosis values both with Pharillons and Riou (Table 9). Finally, these two populations did not present significant differences between them (Table 9).

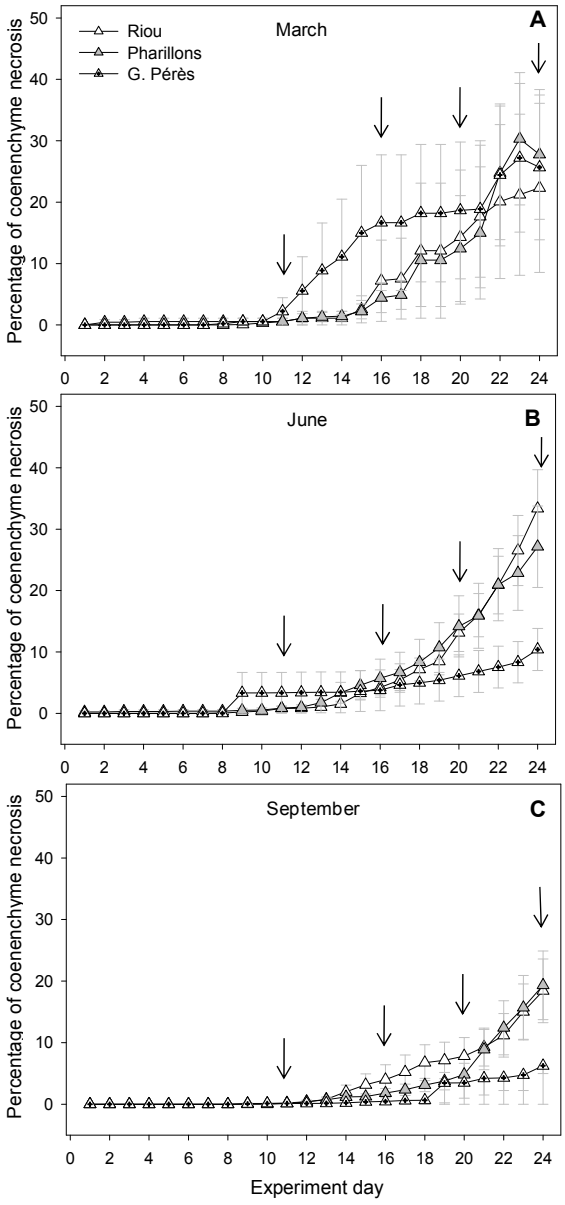

Figure 9. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* Marseille shallow (20 m) populations collected in March (A), June (B) and September (B). Colonies were submitted to a constant T regime of 25°C. Arrows indicate selected days to perform PERMANOVA analyses.

Table 9. PERMANOVA pair-wise comparisons between Marseille shallow populations collected in June (upper-right) and September (lower-left) submitted to 25°C (no statistical differences were found between any pair of sites in March collected populations). ***: p-value<0.001, ** : p-value<0.01, *: p-value<0.05, ns: not significant.

Spet.\June	Riou	Pharillons	G. Pérès
Riou		ns	**
Pharillons	ns		*
G. Pérès	**	*	

3.5. Assessing mass mortality risks and consequences on *P. clavata* populations

Thermotolerance curve versus field temperature conditions. *P. clavata* thermotolerance curve position in relation to field T curves indicated that overall shallow populations are submitted to the temperature conditions close to their thermotolerance limits (Fig 10). Among them, however, populations from Marseille seemed the most vulnerable to suffer damages since it was the nearest to the experimental thermotolerance conditions (Fig. 10), while *P. clavata* shallow populations in Scandola and Medes, but especially in this last area, seemed to be in a less risky position (Fig. 10). Despite, all shallow populations were near the thermotolerance limit when considering Ts equal or greater than 25°C (Fig. 10). Deep populations did not seem to be in a risky position, presenting field T regimes null durations at Ts equal or greater than 25°C (Fig. 10).

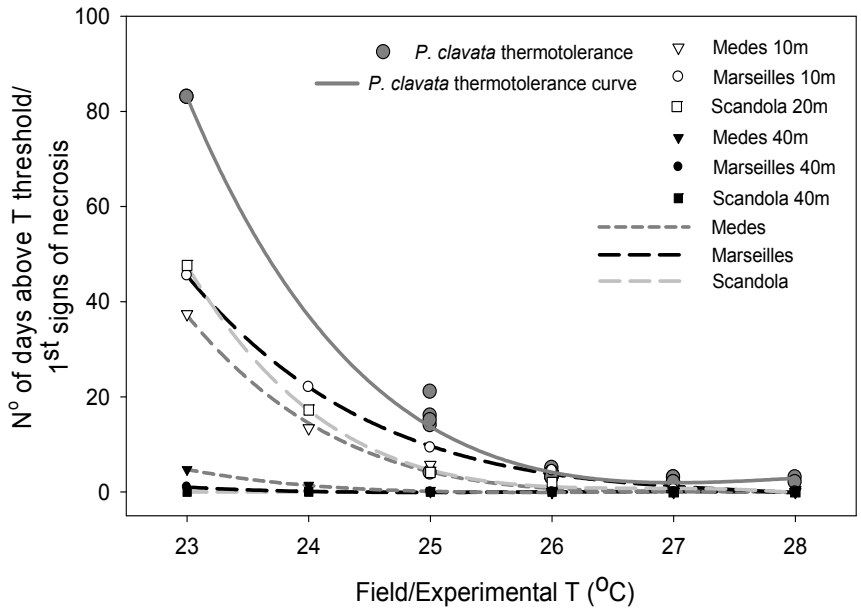

Figure 10. Thermotolerance curve of *P. clavata* (inverse third order regression fitted to experimental data) and field T curves for shallow (10 m for Medes and Marseille, 20 m for Scandola) and deep layers (40 m) obtained fitting a third order regression to highest registered durations inside different Ts.

Percentage of resistant colonies. Regarding the proportion of resistant colonies, intermediate to high percentages of these colonies were found at all studied populations (Fig 11). No clear patterns arose across depths and regions, although beyond 27 ºC all populations showed a clear-cut decrease on resistant colonies.

After 20 days at 25ºC all populations accounted for more than 40% of resistant colonies, presenting most of sites ≈ 80 to ≈ 100% resistant colonies (Fig. 11). When considering the 26ºC T, after 6 days submitted to this T all populations with the exceptions of Medes shallow and deep populations (which although remained with ≈ 20 and ≈ 15% of resistant colonies respectively), presented ≈ 40% or higher percentage of healthy colonies (Fig. 11). After 3 days exposed to 27ºC, all the studied regions (represented by some of its corresponding populations) accounted for ≈ 20% or more resistant colonies (Fig. 11). Finally, after 3 days at 28ºC only ≈10% of resistant colonies remained in Medes shallow population (Fig. 11).

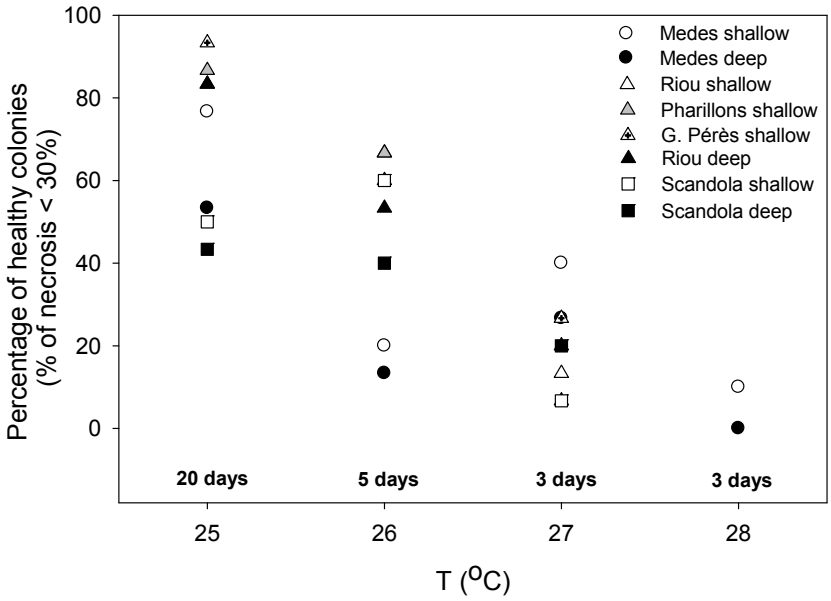

Figure 11. Percentage of resistant colonies (colonies with less than 30% of coenenchyme necrosis) after several days submitted to different experimental T (T2 or T3 of PERMANOVA analyses).

4. Discussion

In this work we addressed for the first time the upper thermal limit of the red gorgonian *P. clavata* and we gave insights in the thermotolerance differences of populations dwelling in regions and depths ranges with contrasted thermal regimes. In addition we investigated the response to T stress of populations collected in different period of the year (winter through summer) associated to differential physiological status of the red gorgonian populations.

Our results suggested that 25°C is a critical T for the studied species because despite the origin, all analyzed populations presented the first's signs of necrosis after some days of exposition to this T. The temperatures of 27 and 28°C were lethal for the species, because all populations suffered extensive mortality rates only after short periods of exposure. At 26°C populations displayed less severe effects and greater inter-population variability than at 27 and 28°C, although all populations showed intermediate to high rates of necrosis after short periods of exposure.

Other studies dealing with thermotolerance features of Mediterranean benthic species showed overall that critical temperatures were also around 25ºC, although inter-specific differences were found. In the case of red coral *Corallium rubrum*, 25°C T was also found critical since analyzed populations showed signs of necrosis after a few days of exposure (Torrents et al., 2008). In the case of symbiotic anthozoans, the white gorgonian *Eunicella singularis* presented a higher resistance to T since the photosynthetic efficiency and calcification rates were not affected until the T reached 26°C (Ferrier-Pages et al., 2009). While the coral *Cladocora caespitosa* showed a decrease in several fitness parameters after long exposures (almost 50 days) to 24°C as well as after several days at 26°C (Rodolfo-Metalpa et al., 2006). This last result illustrates that damaging effects of high T are time dependant and that it is not possible to refer to a single lethal T without specifying the exposure period (Cossins and Bowler, 1987). Furthermore, it has been shown that moderate Ts could be damaging if they are combined with other factors such as starvation (Fitt et al., 2001; Coma et al., 2009).

Precisely, because the effect of T could be exacerbated by other stressing factors, we compared the response to temperature of the same set of populations collected at different periods of the year: winter, spring and late summer. Due biological processes and environmental conditions, physiological status of populations vary significantly between the considered periods being the late summer the most critical period (Coma et al., 1995; Coma and Ribes, 2003; Coma et al., 2009). Therefore, we expected to find an increase of necrosis rates in experiments carried out with colonies collected in late-summer than in other periods.

Contrarily to expected, we did not find statistical differences between periods indicating that physiological status did not modulate populations' response to temperature stress. Despite of this, a pattern arose when inter-population differences within each considered period were examined. Populations exhibited a similar response when the experiment was carried out with colonies collected in winter. Meanwhile significant differences were found between populations collected in late spring and summer. This could indicate that populations rely on developed mechanisms associated to local adaptation or acclimatization processes to environmental conditions found at lower spatial scales than those acting at regional scale (see also below) when the physiological status is low.

In any case, since experiments to test the effect of physiological status on temperature response were carried out with colonies submitted to different number of stabulation days (see Material and Methods), we contend that further experiments should be addressed using similar stabulation periods before concluding on the role of physiological status of populations in modulating the response to T stress.

Thermotolerance response of studied populations was not clearly related to thermal regime (inter-regional and inter-depth analysis) were they dwell. In fact, inter-population level accounted for larger variability than inter-regional or inter-depth levels, e.g. differences within shallow populations from Marseille were in some cases greater than those found between populations dwelling in other regions or depth range. In our case the lack of differences in the response to T of Marseille population during the preliminary experiment suggested the suitability of analyzing one population per region and depth range. However, subsequent experiments revealed significant intra-regional differences (Figs. 6a, 9b, 9c, Table 2 and 7). Previous thermotolerance studies on other Mediterranean gorgonian species (*Corallium rubrum* and *Eunicella singularis*) suggested that populations could be adapted to differential temperature regimes (inter-depth differences) accounting for the observed differences in response to temperature stress (Torrents et al., 2008; Ferrier-Pagés et al., 2009; Ledoux, 2010). However, in contrast to our study, previous experiments did only take into account one population for each temperature regime considered. Therefore, new thermotolerance experiments should consider replication within temperature conditions to test whether or not there are thermotolerance patterns associated to temperature regimes.

Our study demonstrate that populations of the same region displayed differential response to temperature stress indicating that adaptative processes to local conditions (potentially different from temperature regimes) can modulate the response to stress. *P. clavata* populations show a strong genetic population structure at small spatial scales (tens of

meters; Mokhtar-Jamaï et al., 2011) compatible with the development of differential acclimatization capacities and local adaptation processes as observed in the case of red coral (Ledoux et al. 2010a) and other marine species (Johannesson et al., 1993; Hughes and Taylor, 1997; Hughes 1992; Sherman and Ayre 2008; Prada et al., 2008). In this sense, it is worth noting that the population of Grotte Pérès (Marseille region) displayed the lowest impacts. This population is submitted to the influence of the wastewater plume from Cortiou (Burgeot et al., 1996). It is likely that after decades of suffering from pollution, Grotte Pérès population is better "equipped" to support other source of stress than other populations from the same area that not suffer the impact of such strong source of disturbance.

When thermotolerance curves were overlapped to T conditions recorded in the different regions it was easy to observe that shallow *P. clavata* populations live near their upper limit of thermotolerance. While, deep populations are in a less risky position since temperature conditions registered in the field are farther away from the thermotolerance limit of the species. Nevertheless, our experimental results support the observations of differential impact during mass mortality events at intra-population level. Therefore, we can conclude that T conditions alone act as a triggering factor while the final degree of impact would be modulated by other mechanisms such as physiological status, presence of thermo-dependent pathogens and timing of anomalies (Bally and Garrabou, 2007; Coma et al., 2009; Previati et al., 2010; Vezzulli et al.; 2010; Crisci et al., 2011). Besides, an increasing number of mortality reports of deep *P. clavata* populations seem to indicate that lower values of critical T than those found in this study or even other factors could play a major role in triggering mass mortalities events in these depths (Kersting and Linares, 2009)

Since the Mediterranean region is considered a hot-spot for climate change (Diffenbaugh et al., 2007; Coll et al., 2010), it is expected a significant increase in the occurrence of heat waves in the area (Déqué et al., 2007; Diffenbaugh et al., 2007,). In this scenario, shallow *P. clavata* populations from NWM region could be submitted to recurrent episodes of mass mortality events in the next decades. However, recent empirical studies (e.g. Conover and Munch, 2002; Grant and Grant, 2002) attested for interactions between ecological and evolutionary processes on short time frame (Ferrière et al., 2004; Carroll et al., 2007) and showed that anthropogenic selective forces can foster contemporary evolution (i.e. the evolution of heritable traits within populations over tens of generations, Hendry and Kinnison, 1999). Together with contemporary evolution, acclimatization capacity (Wilson and Franklin, 2002) and local adaptation (Williams, 1966; Kawecki and Ebert, 2004) are known to influence population dynamics and evolution. The combination of these three processes

could contribute to mitigate the expected impacts of global change (Skelly et al., 2007; Hendry et al., 2010).

In this sense, most of the analyzed populations showed a relatively high proportion of resistant colonies (Fig 11). Since resistant colonies could participate in reproduction events, their presence may have important implications on acclimatization and/or adaptive capacities to better face the climate change environmental changes counterbalancing thus expected negative impacts. However, we need to consider the interactions between ecological and evolutionary processes especially in the case of long-lived species affected by mass mortality events. In fact, these species are characterized by low dynamics with long generation times and limited dispersal abilities (Lasker, 1991; Yoshioka, 1994; Coma et al., 1998, 2001, 2003, 2004) which could limit the spread of thermo-resistant genotypes. Overall, these biological features could have negative impacts on the adaptive capacity of these species.

Here we showed that thermotolerance experiments can give important clues to assess the response of populations to expected warmer conditions. If we want to anticipate the impacts of climate change, we need to extend the analysis of thermotolerance features to other species covering wide regional scale. Coupling these data to observed temperature conditions as well with expected warming scenarios in the NW Mediterranean (currently under development using the IFREMER Mars3D Model) we could provide a reliable tool to anticipate the effects of climate change in this hot-spot of marine biodiversity.

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Supervised Machine Learning algorithms and their applications to ecological data: a review

CHAPITRE 4

Abstract

Machine Learning (ML) techniques provide a powerful set of statistical tools that deserves serious attention when treating ecological data, which in general do not require the use of simple statistical analysis. In this paper we present a general overview of supervised ML algorithms as well as a brief survey of the ”state of the art” about the applications of these techniques to the particular field of Ecology. In the first part of the paper we introduce the problem indicating the utility of these techniques to treat ecological data. In the second part we describe in a conceptual way the general framework of ML and explain the basis of the underlying theory. In the third part we present some outstanding ML techniques with ecological examples of some of them. Finally, in the fourth part we present a practical example that can be potentially handled with the different proposed techniques. This example refers to the problem of predicting/explaining mass mortality events in the coastal rocky benthic community of the NW Mediterranean Sea from high resolution temperature series. An extensive list of references is given in order to allow the reader to go further and deeper on the discussed topics.

Keywords: Machine Learning; ecological data; Regression Analysis, Classification Rules, prediction, feature selection.

1. Introduction

Ecological systems rarely require simple statistical analysis. For example, much of the data collected by ecologists often exhibit *unusual* distributions, non-linearity, multiple missing values, complex data interactions, dependence on the observations, etc (Fielding, 1999; Guisan et al., 2002; Cutler et al., 2007; De'ath, 2007). The size of data sets is another very hard and frequent problem. Many of the ecological databases are very large and some are continuously expanding. The problems related to a large number of cases or variables are likely to become more severe as more biodiversity data are accumulated and remotely censored data are increasingly used (Fielding, 1999). Bellman's "curse of dimensionality", often invoked by statisticians to refer to how difficult it may be to deal with a huge amount of data, appears nowadays frequently in Ecology.

Machine Learning (ML) techniques are not and never will be the solution to all the problems raised by ecological data. However, these techniques provide a powerful set of tools that deserves a serious attention to deal with some relevant ecological problems. As a first point, let us remark that ML concerns are nothing but the most ancient, classical and widely-studied statistical problems: classification, regression, decision, clustering, density estimation, etc. However, what makes ML a particular field are not precisely its goals and problems but its tools, techniques and strategies, characterized by the massive use of algorithms and computational resources to deal with large sets of data, high number of variables and complex data structures.

Here we intend to present the principles of statistical learning theory and several ML techniques with ecological examples. The approaches we have selected are from our point of view among the best known and most attractive ones. They should be efficient to study a wide panel of ecological problems. Finally we chose to restrict our review to situations where we wish to model the effect of a set of explanatory variables *(X)* on a target variable *(Y)*. This is the context of *Supervised Learning* (SL) said otherwise, *Regression* or *Classification* depending on the nature of *Y*.

ML approaches are intensively applied in different areas and there is no doubt that Ecology is today one of the most relevant areas of ML application (Flach, 2001). This is reflected in the large number of publications that appeared in the last years in which diverse ML techniques are applied to solve a wide variety of problems concerning terrestrial, marine and freshwater ecosystems. Most of the existing works studied the relationship between organisms and environment, such as habitat characteristics and species presence

(Ryder and Irwin, 1987; De'ath and Fabricius, 2000; Guisan et al., 2002; Shan et al., 2006; Cutler et al., 2007), population and community attributes (Defeo and Gómez, 2005; Knudby et al., 2010; De'ath and Fabricius, 2000; De'ath, 2007). Integration of ML techniques and geostatistics led to study the large scale statistical relationship of the distribution of species or communities and their environment (Guisan and Zimmermann, 2000; Benito-Garzon et al., 2006). But other problems such as prediction of algal blooms (Ribeiro and Torgo, 2008), of fish recruitment (Fernandes et al., 2010), organism identification (Morris et al., 2001), land cover mapping over large areas (Rogan et al., 2008) have been analyzed via ML methods.

The paper is organized as follows. In section 2, the general frame work of SL is presented with a glance of its theoretical basis but explained in a conceptual manner. In section 3 we present a wide panel of SL techniques with some ecological examples. We mention some extensions of widely used techniques in particular ecological problems such as prediction of multiclass outputs and probability density functions, and some useful methods for variable selection in high dimensional data framework. Finally we study a specific ecological problem that can be potentially treated with a wide number of techniques among those presented in section 3.

2. Supervised Learning (SL): general framework and fundamentals

The main purpose of SL techniques is to learn how to predict a random variable $Y \in \mathcal{Y}$ based on a set of explicative random variables denoted by $X \in \mathcal{X}$, where $\mathcal{Y}$ and $\mathcal{X}$ depend on the problem at hand but may be thought to be respectively $\mathbb{R}$ and $\mathbb{R}^d$ for example. We will often call X the input and Y the output. As a leading example, one may think about a variable Y that represents the presence/absence of a lichen species and a set of variables X that consists on elevation, aspect and slope (Cutler et al., 2007).

The main problem is to find a predictor

$$f : \mathcal{X} \rightarrow \mathcal{Y}$$

$$X \rightarrow f(X)$$

chosen among the set of all functions $F = \{ f : \mathcal{X} \rightarrow \mathcal{Y} \}$. To build a "good" predictor we have to define a performance criterion that is, a *loss function* denoted by L which depends namely on f, X and Y. We thus say that predictor f is better than predictor g if $L(f,X,Y) < L(g,X,Y)$. To simplify the notation we will omit the dependence of L on X and Y.

Suppose that there exists a unique predictor $f^* \in F$ which minimizes the loss function L, called the *optimal predictor,*

$$f^* = \underset{f \in \mathcal{F}}{Argmin}\ L(f, X, Y).$$

in general however, it is not possible to minimize L over the whole set of possible functions F (that may be a very large set) but only over a given class of predictors C that corresponds to a set of practically computable predictors (for instance the class of linear models). In such a case one obtains a predictor f^{**} satisfying

$$f^{**} = \underset{f \in \mathcal{C}}{Argmin}\ L(f, X, Y).$$

The predictor f^{**} may be different of the globally optimal predictor f^* (which maybe not included in C). Besides, the predictor f^{**} is not available neither in practice, since one is not able to minimize the *loss function* on C but only an empirical version of it $\hat{L}_n$ based on a sample of size n. This empirical estimation corresponds to the predictor used in practice. It is denoted by $\hat{f}_n$ in the sequel, and satisfies

The loss of performance due to the difference between and f^* and f^{**} is of modellistic nature and depends on how relevant is our choice of C (in other words, which model we choose). If a bad choice of C is done, no further sampling will allow balancing this loss of performance. This is why the difference $L(f^*)-L(f^{**})$ is often called *approximation error* or *modeling error*. On the other hand, a second loss of performance, due to the difference between f^{**} and $\hat{f}_n$ is of purely statistical nature. If a very large sample is available (i.e., if n tends to infinity), $\hat{f}_n$ will converge in some sense to f^{**} under suitable hypothesis on our model. This explains why the difference $L(f^{**})- L(\hat{f}_n)$ is often called *estimation error* (Fig. 1). Statistical learning theory aims to give the necessary and sufficient conditions on the class of functions C that would guarantee the consistency of the estimator $\hat{f}_n$, that means its convergence to the optimal predictor f^*.

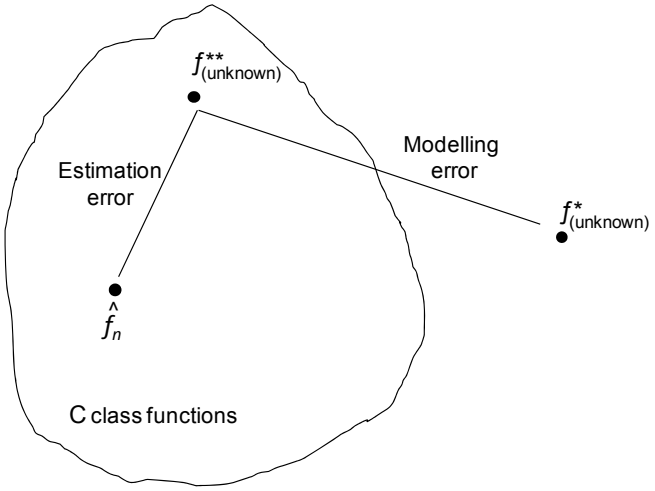

Figure 1. Representation of best of all possible predictors (f^*), best of all possible predictors inside the chosen class *C* of functions (f^{**}) and the empirical predictor ($f^{\wedge}_n$). Adapted from Devroye et al. (1996).

2.1. The optimal model

The loss function used to estimate the model f depends on the nature of *Y*. In regression one generally seeks to minimize the quadratic error:

$$L(f) = E\left[(Y - f(X))^2\right]$$

where *E* is the expectation with respect to the joint distribution of *(X,Y).* In this case the optimal model is the conditional expectation of *Y* over *X*

$$f^*(x) = \underset{f \in \mathcal{F}}{Argmin}\ L(f) = E\left(Y|X = x\right)$$

and the predictor used minimizes the empirical version of the quadratic error,

$$\widehat{f}_n = \underset{f \in \mathcal{C}}{Argmin}\ \widehat{L}_n(f) = \underset{f \in \mathcal{C}}{Argmin}\ \frac{1}{n}\sum_{i=1}^{n}\left[Y_i - f(X_i)\right]^2$$

where $(Xi,Yi)_{i=1..n}$ are *n* independent and identically distributed (*iid*) realizations of *(X,Y).* In classification($Y \in \{1,..,J\}$) the classical loss function is the misclassification error:

$$L(f) = P\left[Y \neq f(X)\right]$$

The optimal model is based on Bayes rule for classification:

$$f^*(x) = \underset{j \in \{1..J\}}{Argmax}\ P[y = j | X = x]$$

where $P[Y = j|X = x]$ is the posterior conditional probability of having level j for Y. Note that in the binary case where $Y \in \{0,1\}$ we have that $P[Y = 1|X = x] = E[Y|X = x]$ and thus the optimal model is given by the conditional expectation just for regression.

In both cases, the choice of class C consists of choosing the mathematical form of f.

2.2. Estimating the model loss

We will generally build the estimator $\hat{f}_n$ of f from a *training sample* $S = \{(X_1, Y_1),..., (X_n, Y_n)\}$. The performance of $\hat{f}_n$ is estimated by means of a new sample $(X_1^v, Y_1^v),..., (X_m^v, Y_m^v)$ called the *evaluation sample*, which is another *iid* sample, independent from the training sample. This method intends to prevent from "overfitting". Indeed, $\hat{f}_n$ has a low error over the learning sample, while its performance may be poor when applied to new data.

Generally, data are given without any precision about the evaluation sample. In practice we randomly split the data at hand in to two subsamples (the learning sample and the evaluation or test sample). The model is estimated over the training set and its performance is studied using the evaluation sample. To reduce any bias due to the random choice of the evaluation sample, the loss is averaged over several random splits of the data (Fig. 2).

Other types of performance estimations, based on well-known procedures such as *cross-validation*, leave one out, bootstrap and other resampling techniques, may be used in practice to give unbiased and numerically efficient estimations. We refer to Hastie et al. (2003) for readers interested in going deeper into the theoretical basis of ML.

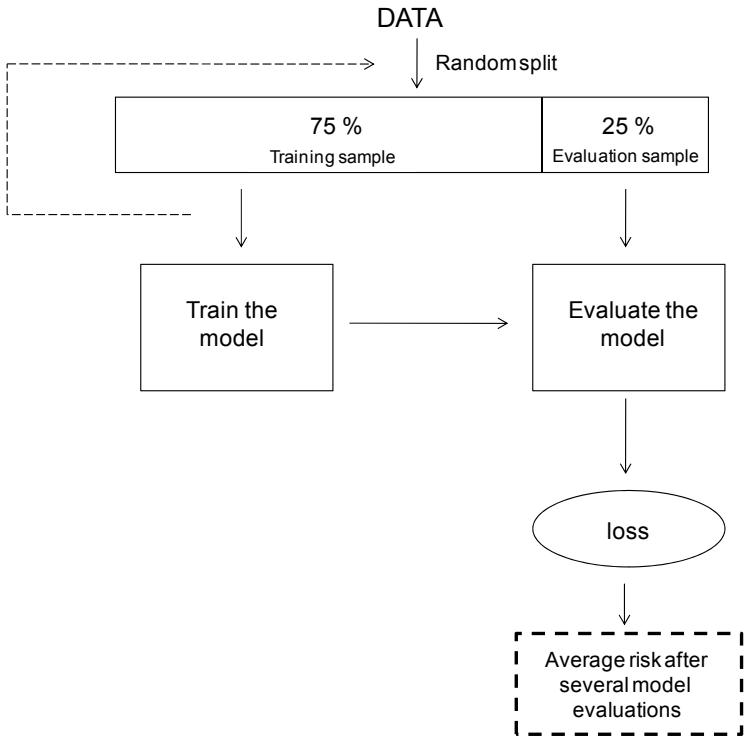

Figure 2. Random data splits for model training and evaluation. To avoid bias estimation due to one random split, several splits are performed and the loss is averaged over the different random test samples.

3. Some particular Machine Learning tools with ecological examples

In what follows we present a short description of some well-known and widely used learning algorithms, with illustrative ecological examples for some of them. Let $X=(X_1,...,X_d) \in \mathbb{R}^d$ and $Y \in \mathcal{Y}$. In case of regression $\mathcal{Y} = \mathbb{R}$ and in case of classification $\mathcal{Y} = \{1,...,J\}$, $J \in \mathbb{N}$. Suppose we have an *iid* sample $S = \{(X_1, Y_1),\ldots,(X_n, Y_n)\}$ where $X_i \in \mathbb{R}^d$ and $Y_i \in \mathcal{Y}$. We denote $x=(x_{\bullet 1},...,x_{\bullet d}) \in \mathbb{R}^d$ a generic realization of X. We present different family of predictors f.

3.1. Generalized additive models

A relevant statistical development of the last thirty years is the advance in regression analysis provided by generalized linear models (GLM, Nelder and Wedderburn, 1972) and generalized additive models (GAM, Hastie and Tibshirani, 1990). These methods provide powerful tools in many scientific research fields among whose ecological research is a good example (Flach, 2001; Guisan et al., 2002).

The traditional linear model, where

$$f(x) = E(Y|X = x) = \alpha_0 + \sum_{k=1}^{d} \alpha_k x_{\bullet k}$$

often fails in explaining ecological data because in real life, effects are seldom linear. Non-linear regression models are more suitable and GAM are among the most practical of them.

GAM may be seen as an extension of GLM which are analogue the of linear models for discrete outputs $Y \in \mathcal{Y} = \{1,..,J\}$. If Y is binary $Y \in \{0,1\}$ and $p(x)= P[Y =1|X = x]$ the GLM has the form:

$$g(p(x)) = \alpha_0 + \sum_{k=1}^{d} \alpha_k x_{\bullet k}$$

where g is a link function which belongs to an exponential family, the most common being the *logit* function $g(p)= \log\left(\frac{p}{1-p}\right)$. For the general case where Y has J levels, we estimate one such model per level and $p(x)$ in the left hand side is replaced by $p_j(x) = P[Y = j|X=x]$.

Additive models generalize the LM by taking the form:

$$f(x) = E(Y|X = x) = \alpha_0 + \sum_{k=1}^{d} f_k(x_{\bullet k})$$

where f_j are typically non parametric univariate smooth models as *Kernel predictors*[1] .

Finally, GAM are the analogous to additive models for discrete outputs:

$$g(p(x)) = \alpha_0 + \sum_{k=1}^{d} f_k(x_{\bullet k})$$

The strength of GAMs is their ability to deal with highly non-linear and non-monotonic relationships between the response and the set of explanatory variables (Guisan et al., 2002). The *d* non parametric functions are simultaneously estimated using efficient algorithms like the backfitting. Note finally that each component f_k could have different form (kernel, splines, etc...).

3.1.1. An ecological example

In a study of the distribution of a supralittoral amphipod in the gradient of dissipative to reflective beaches, the relationship between several physical beach variables and the abundance of the amphipod *Atlantorchestoidea brasiliensis* was analyzed using GAM (Defeo and Gómez, 2005). The output variable was the abundance of *A. brasiliensis* and the input or explanatory variables were physical beach variables: grain size, beach face slope, water content and sand compactation. To avoid including truly redundant variables and yet retaining all important environmental information, the Akaike information criterion (AIC) (Nelder and Wedderburn, 1972) was used in the context of a bidirectional stepwise procedure, allowing to select the most parsimonious model and to assess the significance of each model term. All predictors were included as smooth terms using spline functions (Defeo and Gómez, 2005). Figure 3 shows the estimated contributions of the four explanatory

[1] A Kernel predictor assigns to each value of the input variable *x* a weighted mean of the output variable over all the observations in the sample. Weights are computed using a kernel function which gives low weights for observations which are far from *x* and higher weights for observations close to *x*.

variables introduced in the model.

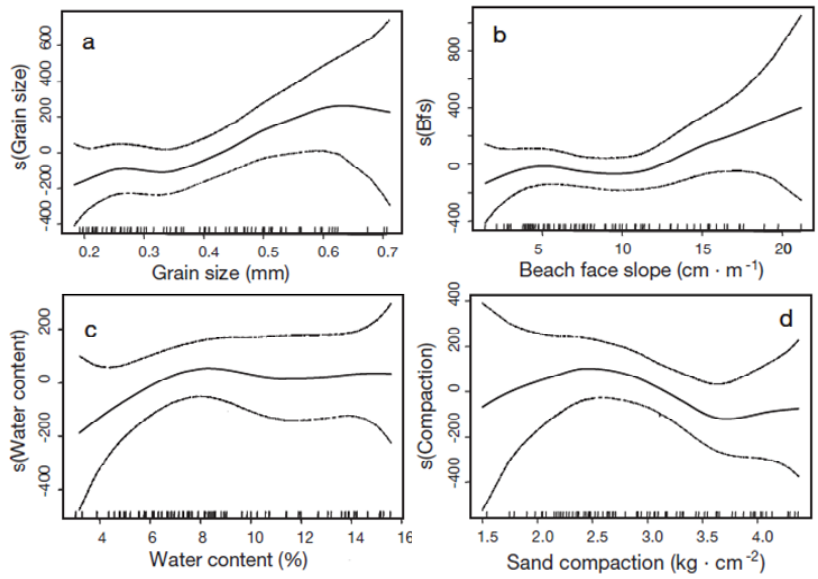

Figure 3. Results of GAM. Smooth functions relating the abundance of *A. brasiliensis* with some explicative variables: (a) grain size, (b) beach face slope, (c) water content and (d) sand compaction. Upper and lower lines indicate the point wise asymptotic standard errors of the estimated curve and the rug plots on the x-axis indicate the range of variables over which measurements were obtained. Taken from Defeo and Gómez (2005).

3.2. Classification and Regression Trees

Classification and Regression Trees (CART), is the most popular of the tree based methods introduced by Breiman et al. (1984). Several extensions of the original method have been proposed (see Loh and Vanichsetakul, 1988; Chaudhuri et al., 1995). The basic idea behind CART is to split the space of explicative variables into multidimensional rectangles, and to use on each of them a very simple local predictor (a constant on each rectangle for instance).

The development of a tree structure comes from the recursive partitioning of the original data set in two subsets that are more *homogeneous* than the first one, leading to a branching structure. The underlying idea is that, as the ramification increases, the *homogeneity* in each node increases too (see Figure 4).

The split of the current data is done via a binary rule over the explanatory variables. For continuous variables the split has the form $x < s$ where s is a threshold over the variable x. When x is discrete, the binary rule takes the form $x \in \mathcal{L}$, where $\mathcal{L}$ is a subset of the possible levels of x. At each step of the partitioning an exhaustive search among all the splits is done. The chosen split is the one that generates the most homogeneous subsamples.

Homogeneity is measured with respect to the target variable *Y*. When *Y* is continuous the criterion is the *deviance*, and we obtain a regression tree. When *Y* is discrete we deal with classification trees and the most used criteria are Entropy and Gini index. In each new subsample (*leaf*), a value of *Y* is assigned. For regression trees, it is the mean value of *Y* over the leaf observations. For classification trees the most frequent level of *Y* in the leaf is used.

Let us now pay attention to the question of when should we stop the ramification. If the number of iterations is small, there will be a lot of data in each terminal node, resulting in low statistical error, but high modelling error. If the number of terminal nodes is large, the contrary happens. Therefore, the optimal number of terminal nodes is an intermediate one (Bell, 1996), and can be obtained by adding to the homogeneity measure of nodes a term that linearly depends on the number of nodes, penalizing the complexity of the tree. This procedure is known as “pruning” and leads to very efficient results.

Finally, let us remark some of the advantages of CART:

- The obtained results are very easy to understand.
- CART is robust to the effects of outliers in the output. Such observations are often isolated into nodes where they do not affect the rest of the tree.
- CART can deal with data sets with complex structures and they are powerful compared with alternative methods as the set complexity in-creases.
- Trees can handle mixed variable types and missing values (Bell, 1996).
- CART gives an importance index for each explanatory variable introduced in the model.
- CART may also be used in the context of Functional Data Analysis, when X is a time series or a signal.

This method has been used in a high number of ecological studies, e.g. species-habitat associations (Bell, 1996; De’ath and Fabricius, 2000; Ryder and Irwin, 1987), species

distributions (Huttmann and Lock, 1997; Ribic and Ainley, 1997), response of species to human impacts (Grubb and Bowerman, 1997).

3.2.1. An ecological example using CART

We chose an example taken from De'ath and Fabricius (2000) to illustrate the use of CART on an ecological problem. The objective was to determine the spatial variables able to explain the presence/absence of the soft coral *Asterospicularia laurae* (De'ath, 2007). Coral abundance and spatial variables were collected during surveys of 374 sites at 92 locations on 32 reefs within Australian central Great Barrier Reef. The spatial variables were: cross - shelf position (inner, mid or outer shelf), within reef location (front, back, channel, flank) and depth (0-1,1-3,3-8,8-13,13-18m) being all of them categorical. The obtained tree is shown on Figure 4. The leaves of the tree are labeled by their dominant category (present or absent). *A. laurae* was absent in inner and mid-shelf reefs (97% of absences so the label is absent). Outer-shelf reefs with back and flank position were also characterized by the absence of *A. laurae* while outer-shelf reefs with front position at depths larger or equal than 3 m were characterized by the presence of the species. For the whole tree 34 of 373 cases were misclassified, leading to a misclassification error of 9.1%.

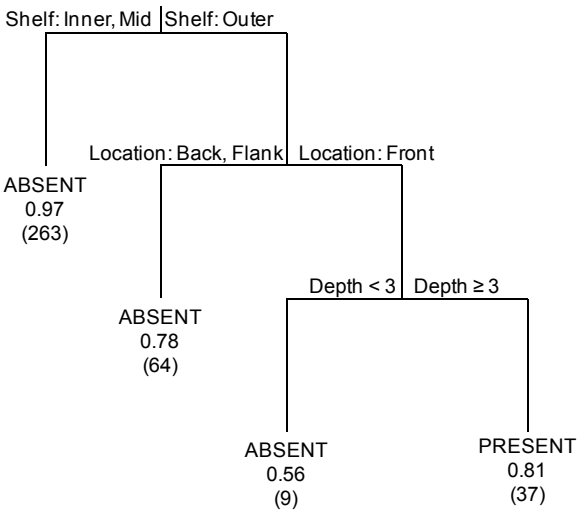

Figure 4. Classification tree for predicting presence-absence of soft coral *A. laurae*. Taken from De'ath (2007).

3.3. Ensemble classifiers

A weak point of CART is its instability with regard to changes in the training sample. A slight perturbation of the learning set may induce important changes in the model structure. Ensemble learning or aggregation techniques arose as a solution to this problem. The underlying idea is to consider a linear combination of several models $f_1,\ldots, f_k$

$$f^{(a)}(x) = \sum_{k=1}^{K} \alpha_k f_k(x)$$

where α_k are real coefficients. If $\sum_{k=1}^{K} \alpha_k = 1$ $f^{(a)}$ is a convex combination. When the output variable Y is discrete $Y \in \{1,...J\}$ the aggregation of $f_1,...f_k$ is generally done using weighted or simply majority vote. Aggregation is generally efficient when the models $\{f_k\}_{k=1,...K}$ are unstable like for instance regression and classification trees. The aggregated model is more stable than any model taken from the ensemble and has generally a lower loss. In what follows we will describe some of these algorithms.

3.3.1. Bagging

Bagging (Breiman, 1996b; Breiman, 1996a) is based on a well-known statistical method called Bootstrap. For a given data set of size *n* a bootstrap sample is obtained by resampling with replacement *n* observations of this data set (Davison and Hinkley, 1997; Efron and Tibshirani, 1994). In Bagging, we generate M bootstrap samples of the training set and perform a CART model on each of them. The final model is obtained by combining the M models. In regression, the bagging predictor is defined as the mean of the M models. In classification, it is defined as the simple majority vote over the M models. Bagging systematically outperforms simple CART models and the gain is generally very significant in regression.

3.3.2. Random Forests

The last and very remarkable contribution of Leo Breiman to the development of ML is the Random Forest technique, based on the use of a large series of low-dimensional regression trees. Its theoretical developments are presented in Breiman (2001). Let us briefly explain the main features of Random Forests in the case of classification. Random Forest performs several classification trees. For a new observation the prediction given by a random forest is a majority vote over the predictions given by the trees in the forest. The trees in the forest

are performed as follows:

- If the size of the training sample is *n*, a bootstrap sample of size *n* will be used to build each tree. This is done in the same way as for bagging.

- No pruning is used: each tree is developed to its largest extent.

- At each node of a tree, the best split is selected among all the splits on only *m* << *d* randomly chosen variables. This avoids the curse of dimensionality and highly increases the speed of the algorithm.

Random Forest presents several noticeable properties: it is probably one of the most efficient learning algorithms in terms of prediction accuracy and it runs fast and efficiently over very large data bases. Besides, it offers an intuitive approach to assess the importance of each explanatory variable used in the model.

3.3.3. Boosting

Boosting is an iterative technique allowing to obtain performant algorithms based on weak predictors or classifiers (see Freund, 1995; Freund and Schapire, 1995). More precisely, one starts with a classifier whose accuracy (probability of success) is just slightly over 0.5, and implements an iterative procedure that requires more efficiency where the previous predictor had the worst performance. In the long run, one will obtain an efficient predictor. There are many variants of this method; the first Boosting algorithm, known as AdaBoost is very clearly exposed in Hastie et al. (2003). AdaBoost was designed for the binary classification problem $Y \in \{0,1\}$. There is a large literature and an extensive work to adapt this algorithm for the regression and to extend it to the multi-class $Y \in \{1,\ldots,J\}$ case. Among the latest works about multi-class extension of boosting algorithm Zhu et al. (2006) gives a very direct and simple algorithm.

3.4. Support Vector Machines

Support Vector Machines (SVM) provide another method of data classification and regression. We will present here only the basic idea of the method, in the context of classification.

Assume that we have a training sample with inputs in R^d and a binary output $Y \in \{-1,1\}$. Assume that the data are *linearly separable* i.e. there exists at least one hyperplane in $\mathbb{R}^d$ which perfectly separates the two subgroups corresponding to each level of Y. We want to select the hyperplane that best separates the classes and which is the farthest possible from all the cases.

Consider an hyperplane H defined by $f(x)=0$ with

$$f(x) = \langle \omega, x \rangle + b$$

where $\omega \in \mathbb{R}^d$ corresponds to the normal vector to the hyperplane, and <,> denotes the dot product in $\mathbb{R}^d$. Note that the sign of $f(x)$ indicates the position of the point x with respect to the hyperplane *H* in $\mathbb{R}^d$. The *margin* of the observation *i* with respect to *H* is defined by:

$$\gamma_i = y_i f(x_i)$$

The margin may be seen as the signed distance to *H*. For an observation *i*,$\gamma i > 0$ if and only if that observation is well classified by *H*. The Hyperplane margin with respect to the sample *S* is defined by $\gamma_H = \min_{i=1,\ldots n} \gamma i$. It corresponds to the margin of the nearest point to *H* (Fig. 5).

SVM aim to find among all the hyperplanes in $\mathbb{R}^d$ the one that maximizes the margin,

$$H^* = \underset{H}{Argmax}\ \gamma_H$$

In the linearly separable case the optimal hyperplane *H** is found using numerical optimization where γ_H is maximized under the constraints that all the observations in the sample are well classified, i.e. $\gamma_i > 0$, $i = 1,\ldots,n$. This hyperplane is unique.

For the non linearly separable case the data are embedded into a space of higher dimension called the feature space using a Kernel function. The underlying idea is that in higher dimension classes may separate more easily (Fig. 6). The Kernel characterizes the transformations of the data.

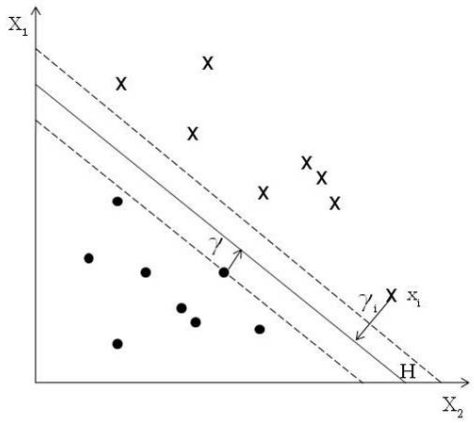

Figure 5. Two linearly separable classes in R^2, γ_i is the margin of observation *i*, γ is the margin of the hyperplane with respect to the sample.

Typically, one uses a Gaussian Kernel;

$$K(x,y) = exp^{-\delta\|x-y\|^2}$$

where $\delta > 0$. For a detailed exposition of SVM see Hastie et al. (2003) and Vapnik (1998).

3.5. Projection Pursuit (in particular, Neural Networks).

The Projection Pursuit Regression model (PPR) maybe written as follows:

$$f(x) = \sum_{m=1}^{M} g_m(< w_m, x >)$$

where $g_1,...,g_M$ are non-specified smooth functions, and ω_m are *d*-dimensional vectors.

Observe that $< \omega_m, x >$ corresponds to the projection of *x* into the direction wm and the model is specified once the *M* directions and functions are estimated. This explains the name of the method: one looks for the best directions, where the more significant features of the input are revealed, and, in that direction, a general non-linear regressor is used (Friedman and Stuetzle, 1981). The function $g_m(<\omega_m, x >)$ is usually called a ridge function.

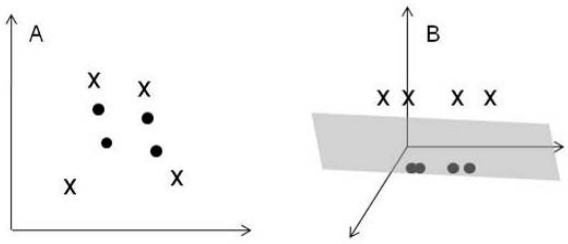

Figure 6. SVM for classification when the two classes are not linearly separable. It two dimensions classes are not linearly separable (A) while at three dimensions there is an hyperplane that separate both classes (B).

From a practical point of view, PPR models are fitted via an iterative procedure that is described in Hastie et al. (2003). When instead of a non parametrical choice of the functions gm, one looks into a particular class of parametric functions, PPR reduces to Neural Networks, probably one of the most widely known learning algorithms (Rosenblatt, 1962; Dreyfus, 2005; Taylor, 2006).

3.6. Nearest Neighbors

Nearest neighbors approaches are among the simplest and most intuitive methods. To compute the predictor $\hat{f}(x)$ at a point *x*, we have to define a neighborhood $N_k(x)$ corresponding to the set of the *k* closest observations to x among the learning sample in $\mathbb{R}^d$. For regression, the predictor is the average of the output over the *k* nearest neighbors,

$$\widehat{f}(x) = \frac{1}{k} \sum_{i \in N_k(x)} Y_i$$

In classification the mean is replaced by the majority vote.

This is clearly a non-smooth method. In binary classification the border line between the two classes is in general a very irregular (Hastie et al., 2003). The *k*-nearest neighbors (*knn*) is robust against outliers. The only unknown parameter is *k*.

3.7. High dimensions and feature selection

Although the *curse of dimensionality* problem is well known in statistics since long ago it appeared again in statistical learning and in several applications where very few observations are available in a very high dimension.

The most known case is that of microarray data where only tens of observations are available with thousands of variables, the genes. Such cases are interesting because classical results in statistics often assume the sample size tends to infinity. Several approaches try to avoid the dimensionality problem by selecting a subset of variables among the original ones. If $X \in \mathbb{R}^d$ one may seek to select among the d dimensions $d' << d$ ones, optimizing a given criterion.

This is known to be a hard combinatorial problem, called feature selection in statistical learning. Reducing dimensionality may reduce noise in the model and obviously the number of measurements necessary for future experiments.

We will briefly present some recent approaches to this problem.

3.7.1. Stepwise procedures

Among the oldest approaches trying to answer the question of selecting the best subset of variables among those available appear the stepwise and its variants, like *Floating Forward* or *Backward Stepwise Selection*. Such approaches stay still computationally expensive and giving often results depending on the order the variables are presented to the algorithm (Kohavi and John, 1997).

3.7.2. Variable ranking and sequential selection

Here one precedes using two steps which may be combined in several manners. First variables are ranked, based on a specified learning model or statistical testing. Then, successive models are adjusted to the data and their accuracy is computed. At each step the least important variables are eliminated from the current model. The best model with regard to predictive accuracy is selected.

Among the most known techniques we may cite SVM *Recursive Feature Elimination* and some of its variants found in Guyon and Elisseff (2003).

3.7.3. Penalization

Penalization appears in statistics even in the most elementary models and it is used in general to avoid overfitting.

It may be seen as a feature selection approach integrated to the model estimation. The optimized loss function is in general augmented by a penalty term whose shape constrains the model to have some desired properties. Thus, such approach is highly related to constrained optimization and depends fully on the used model. For instance, in CART selecting the best subtree from the maximal tree is done by penalizing the optimized criterion used to estimate the model. The penalty term in this case is proportional to the number of leaves in the tree.

For linear and Generalized linear models one may refer to the Least Absolute Shrinkage and Selection Operator (LASSO, Tibshirani, 1996), Least Angle Regression (LARS, Efron et al., 2004) and GLMPath (Park and Hastie, 2006). For SVM a regularized version exists and is called L-SVM (Hastie et al., 2004).

3.8. Extending learning algorithms to specific or general cases

We mention here some of the most challenging extensions for the algorithms described above.

3.8.1. Multi-class extensions

It may be noted that some learning algorithms are initially designed for specific cases like SVM or boosting whose first versions where created for the binary supervised case, e.g. the dependent variable may have only two possible values. An extensive research has been dedicated to extend these algorithms to the *multi-class* case. For SVM we may cite Phetkaew et al. (2003) and for Boosting we refer to Freund and Schapire (1995) and Zhu et al. (2006). Theoretical problems concerning these approaches are still unresolved. The most common approaches generally try to decompose the multi-class problem in to several binary problems, resolve each of these simple problems and aggregate their solutions

3.8.2. Multivariate discrete or continuous output

We may often be concerned by the prediction of a multivariate output $Y \in \mathbb{R}^q$. For instance, ozone observations may be available simultaneously at several q measurement sites. The

different sites may share or not the same independent variables. We would like to use a model to predict the future values of *Y*. One solution would be to adjust one model per site. This solution is not convenient if the observations among sites are dependent. Very few statistical tools provide a direct modelling issue for this situation. For both discrete and continuous multivariate output, there exist extensions of CART model to handle these cases (Zhang, 1998; Segal, 1992). These extensions focus mainly on adapting the splitting criterion of CART to the multivariate case, and finding convenient estimators for the criterion at each step of the tree construction.

3.8.3. Functional data

When either the output variable *Y* or one of the explanatory variables may be seen as discretized versions of a continuous curve, one may need functional data analysis (FDA) tools (Ramsay and Silverman, 2002). FDA approaches have appeared in the early 90's and have extended several classical data analysis techniques (Principal Component Analysis, Regression, Discriminant Analysis, Analysis of variance,…) to the functional case. The main idea of these approaches is to construct a mathematical representation of each observed curve in a functional space using a common basis, like splines or wavelets. FDA approaches have been integrated to some Supervised Learning techniques. For instance, Nerini and Ghattas (2007) extended CART to the case where Y is a density curve discretized at *q* points. The extension they proposed may handle also the case where each observed curve is discretized at different points. Their application concerned the prediction of the zooplankton size distributions using wind and temperature measurements. For independent functional data, very few works appear in the literature. In particular there exists an extension of CART to the case where the independent variable is supposed to be a time series (Yuu et al., 2003). In this work FDA approaches are not used. The underlying idea consists on adapting the splitting rule which has initially the form $x_j < s$. The splitting rules become $D(x(t),x_0(t)) < s$ where $x(t)$ is one explanatory functional independent variable (observed temperatures over one day for example), $x_0(t)$ is one of the observations present in the learning sample, s is a threshold and D is a dissimilarity measure between two time series. The dissimilarity measure is based on *Dynamic Time Warping* and computed directly over the original data.

4. A detailed example

In this section we briefly expose an ecological problem that may be studied by means of different ML techniques abovementioned, depending on the questions one would like to address. We give some receipts and guidelines to use suitable models among those described in the previous section, counting also for the possible preprocessing of the data available for this example.

4.1. Modelling mortality events in the coastal rocky benthic communities

In the lasts years, mass mortality events of unprecedented extension and severity were registered in the coastal rocky benthic communities of the North- Western (NW) Mediterranean Sea during the summer period. Long-lived organisms such as gorgonians were within the most affected (Cerrano et al., 2000; Perez et al., 2000; Garrabou et al., 2009). These mortality events were related to positive thermal anomalies occurring during summer (Bensoussan et al., 2010). Since warming trends were detected for the NW Mediterranean coastal waters (Romano et al., 2010), future mass mortality events are expected to occur.

The main objective here is both to understand the role of temperature in these events and to generate predictive tools for future mortality episodes.

4.2. The data

Data were collected from four regions of the NW Mediterranean sea:

- Parc Natural del Montgr´ı, Illes Medes i Baix Ter, L'Estartit, Catalonia, Spain,
- Riou, Marseille, France,
- Parc National de Port-Cros, France,
- Reserve Naturelle de Scandola, Corsica, France.

On one side, biological surveys were carried out at each region from shallow (≈ 5 m) to deep waters (≈ 40 m) in the 1999-2006 perdiod (although not all regions were surveyed all years). Several sites were surveyed inside each of the four study regions, the number of sites varies from 2 to 14 depending on the region and the year. Inside each site the mortality of a

minimum of 70 colonies was registered for 1 to 3 gorgonian species (*Corallium rubrum*, *Eunicella cavolinii*, *Paramuricea clavata*). In total, more than 20.000 colonies were surveyed. For each colony, mortality was computed as the proportion of the colony affected either by recent epibiosis or denuded axis (skeleton).

On the other side, at each region thermometers were placed from 5 to 40 meters depth every 5 meters to give hourly measurements. Data are available from 1999 to 2006 at Riou and Port-Cros, but measurements began on 2002 for Illes Medes and on 2004 for Scandola.

The two greatest mortality events were observed during summers 1999 and 2003 and a less severe one was observed during summer 2006. Hence temperature data for each of these events are available on at least two regions.

4.3. Guidelines for ML methods to study the temperature -mortality relationship

The dependent variable is mortality. It may be seen as a function of the following explanatory variables:

- the raw temperature series,
- the Year,
- the Region
- the Species and
- the Depth.

Overall, the explicative variables are of different natures: continuous and categorical (ordinal and nominal). Only temperature is continuous and varies as a function of Year, Depth and Region. To simplify the presentation of the possible modeling approaches we will consider that each observation corresponds to one site. Thus we study possible models of the form:

$$Y_s = f(year, region, species, depth, T)$$

where T is the temperature and Y_s is the mortality observed at site *s*. As for each site the number of colonies varies from 70 to 400, each observation Y_s has a different length.

4.3.1. Accounting for the nature of the output variable

Different choices of models are possible according to the preprocessing of the mortality observations within a site.

- One may take the average of mortality over all the colonies within the site. Any other statistic like quantiles or extremes may be used leading to a unidimensional continuous output. Almost all the models described in the previous section may be used in this case.
- One may also produce a new multidimensional output vector by grouping several statistics computed over the colonies of the same site. In this case only multivariate output regression models can be used and especially, the corresponding extension of CART described in 3.8.2.
- Finally, one can compute an estimation of the cumulative distribution function, or the distribution function evaluated at a fixed grid of points and use an approach allowing to predict functional output like in 3.8.3

4.3.2. Accounting for the nature of the independent variables

Although several models can handle categorical variables, some of them, like classification and regression trees, are particularly suitable and efficient. In our case the only continuous variable is temperature and it appears to be functional for each site. Recall that for each depth, year and region, temperature is observed hourly during the summer leading to almost 3x30x24 =2160 measurements per observation. Different preprocessing of the temperature data may lead to different modelling options:

The first option is to use temperature including each hourly measurement as a variable. This leads to a very important number of correlated variables. Although this option is seldom chosen in practice, one might in this case use regularized models such as those cited in 3.7.3.

We can obtain temperature predictors by extracting different statistics from the series (such as maximum, mean, coefficient of variation) at different time scales (whole summer period, each month, any period time of fixed length, etc...). Other variables related to the moment of the summer where warmest events occurred may be of interest since it appears that at different moments of the summer, organisms present different physiological status (Coma et al., 2009).

Finally, we may keep temperature as a functional independent variable and in this case use a supervised learning model able to handle such variable, as the one described in section 3.8.3.

If we rather focus on a prediction task, it should be noted that the temperature used in any of the three cases should be taken either with one year decay, or replaced by an estimate built on a statistical model (based for instance on time series analysis).

It is important to note that when different models are possible some might be more useful to understand the relationship between mortality and temperature, while others might be more accurate for prediction task. For example, CART and GAM methods have very clear outputs, which makes them easy to interpret but they can fail in making accurate predictions. CART is known to be unstable with respect to little changes in the data. On the other hand, GAM does not work very well in presence of correlated predictors. To make accurate predictions models with more complex structure are in general more appropriate, but their interpretation becomes more difficult. Bagging, Random Forest, SVM, Projection Pursuit are in this last category.

It is clear that in many cases it is desirable to understand the problem and at the same time have accurate results, as in the case of our example, in which we want to develop a powerful tool to generate prediction but also to know which temperature attributes are most related with organisms death. The best model will generally depend on the problem, on the sample at hand as well as on the preprocessing of the data. In general, several models are tested and compared using reliable estimates of the prediction errors.

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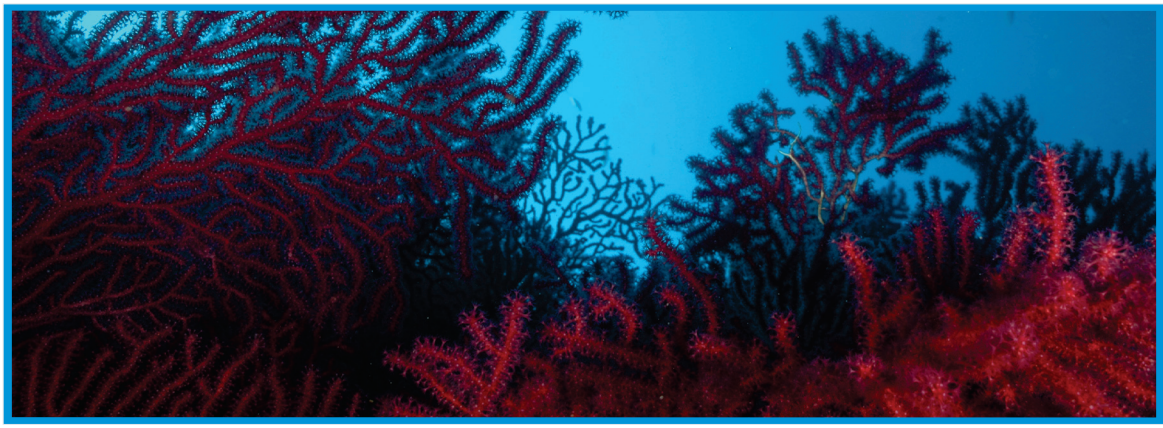
Prediction of mass mortality events in the NW Mediterranean rocky benthic communities through high resolution temperature data using Supervised Learning techniques



CHAPITRE 5

Abstract

The acquisition of high resolution temperature series and biological data during years with and without mass mortality events (MME) in the rocky benthic community of the NW Mediterranean (NWM), presents for the first time the opportunity to generate statistical predictive models of mortality outbreaks. For this purpose, we chose several Supervised Learning (SL) models, in particular, Classification and Regression Tress (CART), Bagging and Random Forest. CART is characterized for providing an interpretable output while Bagging and Random Forest are reported as very accurate predictors. Mortality of three gorgonian species at four regions of the NWM was considered for the 1999-2006 period at the 10-40 m depth rank. Two mortality indicators were selected as response variables: The percentage of colonies with ≥ 10% (nec_10) and with 100% (nec_100) of tissue necrosis inside a given site, year and depth. A total of 23 temperature descriptors were considered as predictive variables. These variables consider temperature magnitude and variability at different time scales of the summer period and also the timing of hottest episodes. For each of the response variables, the three different models gave similar error rates. When modeling the nec_10 variables the error was of aproximately 9%, while when considering the nec_100 variable it was of near 4%. When testing the models with a new data set (that of the year 2006) the three models presented errors of ~5% and ~0.5% for the nec_10 and nec_100 variable respectively. This suggested the suitability of the performed models to predict mortality from a new set of temperature data. Because of the great number of predictive variables, models reduction was carried out considering some the most important variables computed using the Forest model. The reduced models gave very similar errors than the models considering all variables. These simpler models may be kept to perform future predictions. For the nec_10 variable the models predicted good for intermediate to high values of this variable, although we observed some over and underestimations. Models with the nec_100 response in general correctly predicted the highest values of the variable. Models inaccuracies could be in part explained by the high variability of the biological response observed during the MME. The acquisition of future biological and temperature data is of great importance for improve models predictions. At the same time, modeling of future temperature scenarios for different regions of the NWM coastal waters is crucial to use modeled temperature to predict mortality.

Key words: predictive models; Supervised Learning techniques; high resolution temperature series; mass mortality events; temperature anomalies; climate change.

1. Introduction

Global warming is an emerging threat to ecosystems worldwide (Salomon et al., 2007). Marine coastal environments are suffering dramatic increases in mass mortality events (MME) and diseases associated to ocean warming (Harvell et al., 1999, Harvell et al., 2002). In particular, in the NW Mediterranean (NWM) Sea, two unprecedented mass mortality events of sessile macrobenthic invertebrates were observed during 1999 and 2003 (Perez et al., 2000; Garrabou et al., 2009). These events covered an extensive geographical area (several thousand kilometers of coastline) and involved wide range of species (approximately 30 species from 5 phyla). Gorgonians and sponges were within the most affected organisms g (Perez et al., 2000; Garrabou et al., 2009). Observed mortality outbreaks were associated with anomalous high temperature conditions during the summer period (Garrabou et al., 2009; Bensoussan et al., 2010; Chapter 2). In particular, the 1999 MME was characterized by long periods (near one month) at warm mean temperatures (~24^{o}C) associated with a low variability in the thermal regime. On the other side the 2003 MME presented short episodes (2-5 days of duration) with high mean temperature (which were in some regions near 27^{o}C) and high variability of the thermal region inside these hot periods (Chapter 2, Crisci et al., 2011).

The presence of abnormal temperatures during MME, associated with the detection of a warming trend in the NWM (Romano & Lugrezi, 2007, Bensoussan et al., 2009) raised concern in the scientific community about the potential effects of global warming on Mediterranean diversity (Bianchi, 2007; Garrabou et al., 2009).

Previous works characterized mass mortality events (Perez et al., 2000; Linares et al., 2005; Garrabou et al., 2009) in the NWM region. At the same time, other studies characterized summer thermal regimes of different NWM regions within years with and without MME (Bensoussan et al., 2010). Finally, further works analyzed the variability of the mortality pattern among regions, years and depths and its relation with summer temperature regimes (Chapter 2; Crisci et al., 2011). But, at the moment, no statistical models were performed to generate a predictive tool of mortality based on temperature data. The combination of acquired high resolution temperature time series and of biological data during years with and without mortality events in different regions of the coastal waters of the NWM, gives for the first time this opportunity.

Supervised Learning (SL) techniques are effective statistical models that can deal with the most usual problems that appear when analyzing ecological data (e.g. high dimensional data, nonlinear relation between variables, missing values, complex data structure, Fielding,

1999, De'ath, and Fabricius, 2000). These techniques cover a wide class of statistical tools such as classification and regression; they use intensive algorithms and often give accurate results.

In this work we use several SL techniques to assess the relationship between summer thermal regime and mortality impacts in the NWM rocky benthic community and to provide a predictive tool for mortality events.

The integration of predicted temperature of the NW Mediterranean coastal waters combined with the models predictions performed in this work could provide a practical tool to generate risk maps of occurrence of MME in different regions of the NWM.

2. Materials and Methods

2.1. Study region

The study was carried out in four locations of the NW Mediterranean basin, being from west to east: Parc Natural del Montgrí, Illes Medes i Baix Ter (L'Estartit, Spain); Riou (Marseille, France); Parc National de Port-Cros (France) and Reserve Naturelle de Scandola (Corsica, France) (Fig. 1). These sites present different hydrological conditions and hence contrasted thermal regimes. A detailed description of the thermal characteristics of each region can be found in Bensoussan et al., 2010, Chapter 2 and Annex 2.

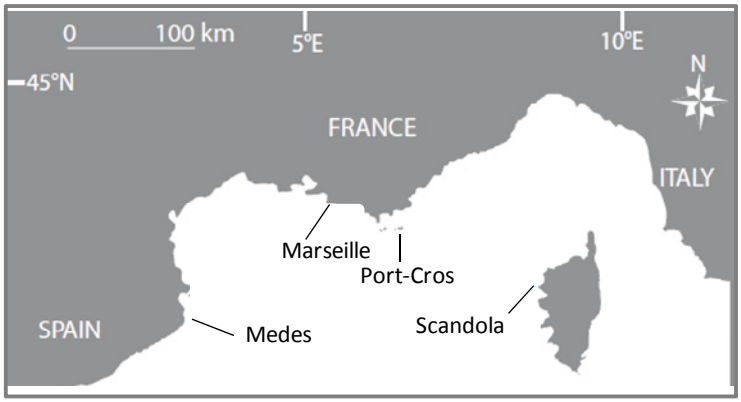


Figure 1. The Northwestern Mediterranean region with the four study areas.

2.2. Temperature data

In each region, temperature records were registered by *in situ* by Stowaway Tidbits autonomous sensors set up in sites exposed to dominant winds and currents. The study period was from 1999 to 2006. Records began in June 1999 at Riou and Port-Cros, in July 2002 at Medes and in April 2004 at Scandola. Inside each region records were performed hourly from 5 to 40 m depth each 5 m. The data analyzed in this work concern only the summer period (arbitrarily set between the 1st July and the 30 September).

2.3. Biological data

Biological surveys were conducted after the summer period in the four study regions. Mortality was estimated as the percentage of tissue (coenenchyme) loss (or necrosis) of each colony (Fig. 2) i.e. the proportion of the surface of each colony that showed no tissue or overgrowth by other organisms, including both affected and unaffected colonies. The overgrowth of other organisms (epibiosis) was considered in the percentage of tissue loss

only when pioneer epibionts were present (hydroids and filamentous algae) (Linares et al., 2005). This was done intending to avoid registering older mortality (previous to the mortality event).

Figure 2. Examples of rates of tissue necrosis in gorgonian colonies. Taken form Perez et al., 2000.

Percentage of tissue loss was quantified by scuba divers in at least 70 colonies per species at each site and depth surveyed. Surveys were performed for three gorgonians species: *Corallium rubrum*, *Eunicella cavolinii* and *Paramuricea clavata.* These species are important structural and biomass contributors of the coralligenous communities, one of the most diverse communities of the Mediterranean (Ballesteros, 2006). Thus, mortality models for these species can be generalized to other species of these communities.

Survey periods, regions and depths differed from one species to another (for example, *C. rubrum* surveys were only conducted in Marseille region during 2003, see Table 1). Surveys were conducted every year at 2-14 sites within each study area and concerned the 10 - 40 m depth range. In Port-Cros and Scandola, there were no surveys in shallow waters (20 m or shallower) because at shallow depths populations of studied species are absent or show low abundance (Linares et al., 2005). When a MME was observed, the number of surveyed sites was increased when possible to better quantify the mass mortality impacts; this explains the great variability in the number of sites (2 to 14). In total, more than 20.000 colonies were analyzed. Table 1 summarizes the available biological data.

Table 1. Biological data availability at each region and year. Surveyed depths and species and the number of sites where data were registered are indicated. When more than one species were surveyed at a given region and year, the number of surveyed sites is indicated for each species separated by comas.

Region	Year	Depths	Species	Num. of sites
Medes	2002	10, 35	*P. cla*	3
	2003	10, 35	*P. cla*	4
	2004	10, 35	*P. cla*	4
	2005	15, 30, 35	*P. cla*	6
	2006	15, 30, 35	*P. cla*	4
Marseille	1999	10, 25	*E. cav, P. cla*	2,3
	2001	25, 40	*P. cla*	6
	2003	10, 15, 20, 25	*C. rub, E. cav, P. cla*	5,9,14
	2004	20	*P. cla*	3
	2005	20, 35	*P. cla*	2
	2006	10, 20, 35	*P. cla*	8
Port-Cros	1999	25	*P. cla*	3
	2002	25	*P. cla*	2
	2003	25	*P. cla*	2
	2005	25	*P. cla*	2
	2006	25	*P. cla*	2
Scandola	2004	20	*E. cav, P. cla*	1,4
	2005	20	*E. cav, P. cla*	1,4
	2006	20, 25	*E. cav, P. cla*	4,4

2.4. Response and predictive variables

Two response variables were selected to test separately their relationship with predictive variables. We computed the response variable at the *site* level instead of considering the mortality data at the colony level. This avoids from replicating temperature data (what would compromise model fit).

The analyzed response variables were:

- **Percentage of colonies with necrosis ≥ 10% (nec_10 from now on):** percentage of colonies with more than 10% of necrosis among the colonies present at one site and depth of a given year. This variable showed to be very useful to quantify mortality and identify inter-regional patterns during the 2003 mortality event (Garrabou et al., 2009).

- **Percentage of colonies with 100% of necrosis (nec_100 from now on):** percentage of colonies with 100% of necrosis among the colonies present at one site and depth of a given year. Although this indicator showed to be more useful to quantify delayed effect of mortality impacts (Linares et al., 2005), it was selected to predict high mortality impacts in the macrobenthic communities since it considers the percentage of totally dead colonies.

Both selected response variables are continuous and unidimensional.

Explanatory variables are presented in Table 2. They consist in temperature variables extracted from the hourly temperature series. They consider temperature magnitude and variability at different time scale of the summer period and also the timing of hottest episodes.

Biological and Temperature data from the same region, year and depth were associated to perform the statistical models.

Previous work demonstrated that summer thermal regime could explain differential mortality impacts among regions, years and depths (see Chapter 2, Crisci et al., 2011). For this reason we did not include the variables Region, Year and Depth in the models, assuming that their importance as predictive variables could be associated with their correlation with temperature variables.

Table 2. Temperature variables used to fit the models. For a detailed description of these variables see Chapter 2.

Variable label	Description
Mean	summer mean T
Max	summer maximum T
CV	summer coefficient of variation (CV)
Mean_CV_5	Average CV of the 5 days with the highest mean T
prop23	proportion of the summer with T in the [23-24[oC interval
prop24	proportion of the summer with T in the [24-25[oC interval
prop25	proportion of the summer with T in the [25-26[oC interval
prop26	proportion of the summer with T in the [26-27[oC interval
prop27	proportion of the summer with T in the [27-28[oC interval
MeanT_2	Mean T of the two summer consecutive days with the highest mean T
CV2	CV of the two summer consecutive days with the highest mean T
MeanT_5	Mean T of the five summer consecutive days with the highest mean T
CV5	CV of the five summer consecutive days with the highest mean T
MeanT_10	Mean T of the ten summer consecutive days with the highest mean T
CV10	CV of the ten summer consecutive days with the highest mean T
MeanT_15	Mean T of the 15 summer consecutive days with the highest mean T
CV15	CV of the 15 summer consecutive days with the highest mean T
Tim_15	Timing of the 15 summer consecutive days with the highest mean T
MeanT_30	Mean T of the 30 summer consecutive days with the highest mean T
CV30	CV of the 30 summer consecutive days with the highest mean T
MeanT_40	Mean T of the 40 summer consecutive days with the highest mean T
CV40	CV of the 40 summer consecutive days with the highest mean T
Tim_40	Timing of the 15 summer consecutive days with the highest mean T

The variable Species was not included neither due to unequal representation of different species among different regions and years (see section 2.3); this variable could potentially hide the importance of temperature variables associated to the year/regions where a given species was collected. We considered that performing the analysis with all the species together is a good approximation since previous studies of the MME in the NWM indicated similar mortality patterns among different species studied here (Perez et al., 2000; Garrabou et al., 2009).

In Figure 3, the outline of temperature and biological data availability is presented.

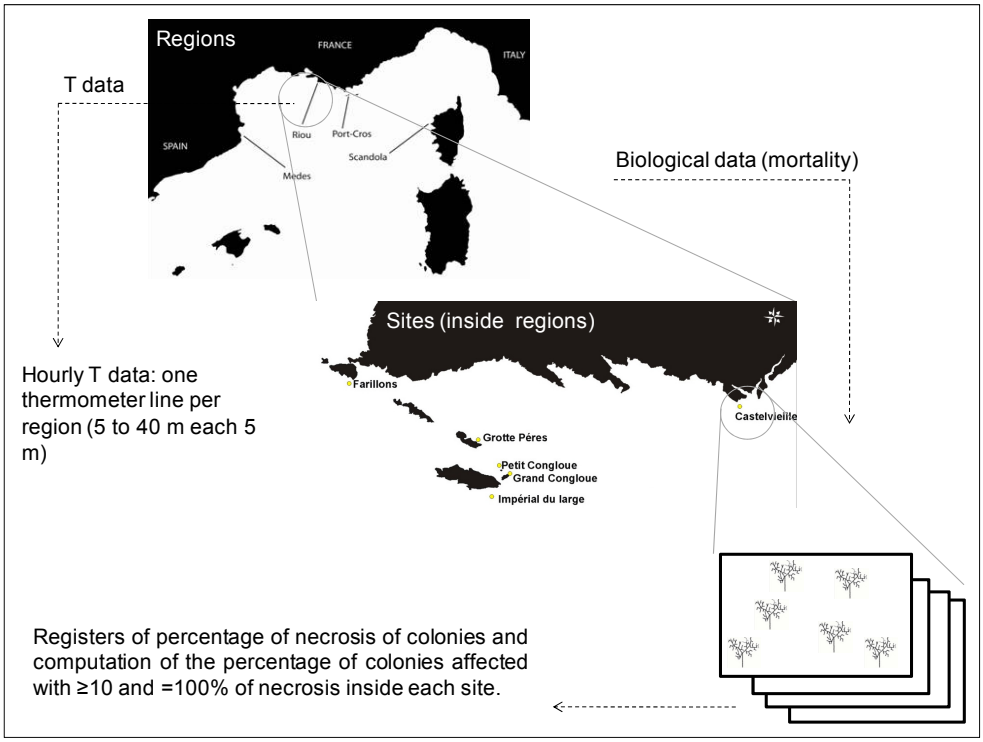


Figure 3. Outline of temperature and biological data surveys. One thermometer line was placed at each study region (temperature sensors from 5 to 40 m depth each 5 m). Inside each region, several sites were surveyed to acquire biological data. With the data of all surveyed colonies of a given species, at each site the percentage of colonies with ≥10% and 100% of necrosis was computed.

2.5. Supervised Learning (SL) models

Among the Supervised Learning techniques, the three following models were chosen to study the relation between the mortality impacts observed in the rocky benthic community and the summer thermal regime:

- **Classification and Regression Trees** (Breiman et al., 1984),
- **Bagging** (Breiman, 1996a and 1996b),
- **Random Forest** (Breiman, 2001).

CART has several advantages; mainly it gives an interpretable output. Bagging and Random Forest are reported as very accurate predictors. In addition any of these models can handle variables of different nature, for example, with continuous and discrete inputs (predictive variables) and outputs (response variables). Finally, both CART and Random Forest are useful to asses variables importance. This feature is crucial because selecting important inputs should lead to simpler predictive models. For a detailed description of these models, their application to ecological data and their advantages and constraints see Chapter 4.

We compared the three models performance using their quadratic errors, defined as:

$$error = \sqrt{(mean(predicted - observed)^2)}$$

2.6. Model implementation

2.6.1. Model form

The performed models have the general form:

$$y_s = f(T) + \mathcal{E}$$

where T is the temperature (considering all temperature variables extracted from the series), y_s is the mortality observed at a site *s*, *f* is the predictor and $\mathcal{E}$ a random noise.

2.6.2. Testing scenarios

We run two *testing scenarios* for each model and each response variable which differed mainly in the choice of the test sample (Fig. 4).

Testing scenario #1:

The data were randomly split using 2/3 to train and 1/3 to test the models. The model accuracy was averaged over 10 such splitings.

Testing scenario #2:

We trained the models with the 1999-2005 data and evaluated their performance with a new set of temperature data, that of 2006 (the choice of 2006 to test the models was arbitrary).
In practice, to predict mortality for a given year, temperature observations are not available and might be replaced by their predictors. As predictions are not available in our case, we use the observations. Our results are thus expected to be *optimistic.*

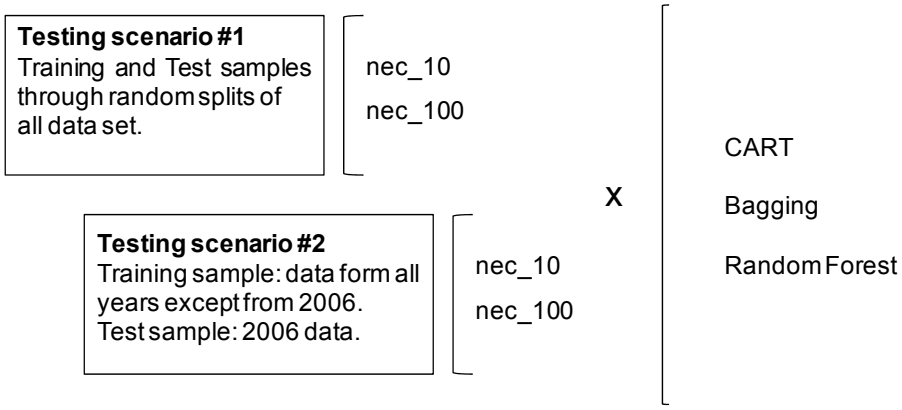


Figure 4. Testing scenarios representation: the two testing scenarios were used to model the two response variables with the three proposed models.

2.7. Models tuning

All models were performed using R software (version 2.10.1). For CART we used *rpart* package and for Random Forest, the *randomForest* package. For Bagging an original *script* was implemented since the available software packages are suitable for classification problems (discrete outputs) and not for regression (continuous output). The script generated to run Bagging is given in Annex 4.

For the three models the defaults values of the parameters were used. For Random Forest variables importance was also computed.

The scripts for testing scenarios #1 and #2 are given in Box1 and Box 2 respectively.

2.8. Variables importance and models reduction

Random Forest importance variables plots were analyzed to retain the most important variables and evaluate models performance using selected variables. Those variables that contributed more than 10% in the Mean Square Error reduction were retained to perform a reduced model. CART representation was also performed to attest for the most important variables provided by the optimal tree (rpart function). Tree plots were also useful because of their clear interpretation of the relation between predictors and response variables.

Finally, after reducing the model, the *predict* function was implemented to asses the predicted mortality rates with both testing scenarios using Random Forest model.

BOX 1. R script for Testing scenario #1.

```
K= 10

x=datos[,17:39]
dep = datos[,5:6]
error=list(matrix(NA, K, 3),matrix(NA, K, 3))
n = nrow(datos)
for(k in 1:K)          {
        smp=sample(n,round(n/3))
        xlearn=x[-smp,]
        ylearn=dep[-smp,]
        xtest=x[smp,]
        ytest=dep[smp,]
        for(i in 1:2)          {
                print(c(k,i))
                ll = cbind(ylearn[,i],xlearn)
                names(ll)[1] = "yy"
                tt = cbind(ytest[,i],xtest)
                names(tt)[1] = "yy"
                resarb = rpart(as.formula(paste("yy~", paste(names(ll[,-1]),collapse="+"))),data=ll)
                prev = predict(resarb,newdata=tt)
                error[[i]][k,1] = sqrt(mean( (prev - tt[,1])^2))
                resbagg =bagging.reg(as.formula(paste("yy~", paste(names(ll[,-1]),collapse="+"))),data=ll,maxdepth=10,mfinal=250)
                prev = predict(resbagg,newdata=tt)
                error[[i]][k,2] = sqrt(mean( (prev$class - tt[,1])^2))
                resrf=randomForest(as.formula(paste("yy~", paste(names(ll[,-1]), collapse="+"))),ntree=250,na.action=na.omit,importance=T,data=ll)

                indNA = rowSums(is.na(tt[,-1])) == 0
                prev = predict(resrf,newdata=tt[indNA,])
                error[[i]][k,3] = sqrt(mean( (prev - tt[indNA,1])^2))


        }
}
```

BOX 2. R script for Testing scenario #2 .

```
K=1

error=list(matrix(NA, K, 3),matrix(NA, K, 3))
n = nrow(datos)
for(k in 1:K)          {
        xlearn=datos[1:80,17:39]
        ylearn=datos[1:80,5:6]
        xtest=datos[81:102,17:39]
        ytest=datos[81:102,5:6]
        for(i in 1:2)          {
                print(c(k,i))
                ll = cbind(ylearn[,i],xlearn)
                names(ll)[1] = "yy"
                tt = cbind(ytest[,i],xtest)
                names(tt)[1] = "yy"
                resarb = rpart(as.formula(paste("yy~", paste(names(ll[,-1]),collapse="+"))),data=ll)
                prev = predict(resarb,newdata=tt)
                error[[i]][k,1] = sqrt(mean((prev - tt[,1])^2))
                resbagg =bagging.reg(as.formula(paste("yy~", paste(names(ll[,1]),collapse="+"))),data=ll,maxdepth=10,mfinal=250)
                prev = predict(resbagg,newdata=tt)
                error[[i]][k,2] = sqrt(mean( (prev$class - tt[,1])^2))
                resrf=randomForest(as.formula(paste("yy~", paste(names(ll[,-1]), collapse="+"))),na.action=na.omit,importance=T,data=ll)

                indNA = rowSums(is.na(tt[,-1])) == 0
                prev = predict(resrf,newdata=tt[indNA,])
                error[[i]][k,3] = sqrt(mean( (prev - tt[indNA,1])^2))



        }
}
```

3. Results

3.1. Descriptive results

In this section we present descriptive results of the response variables (Fig. 5) as well as mortality relation with Depth and Year (variables that were not included in the models, see Material and Methods section, Figs. 6 and 7). The relation of response variables with explanatory variables is presented at the end of this section only for the most important variables (see section 3.2.3).

3.1.1. Response variables

Distribution of mortality variables is concentrated near zero, indicating that most of the surveyed sites among regions, years, depth and species presented very low percentage of colonies with more than 10% and with 100% of necrosis (Fig. 5).

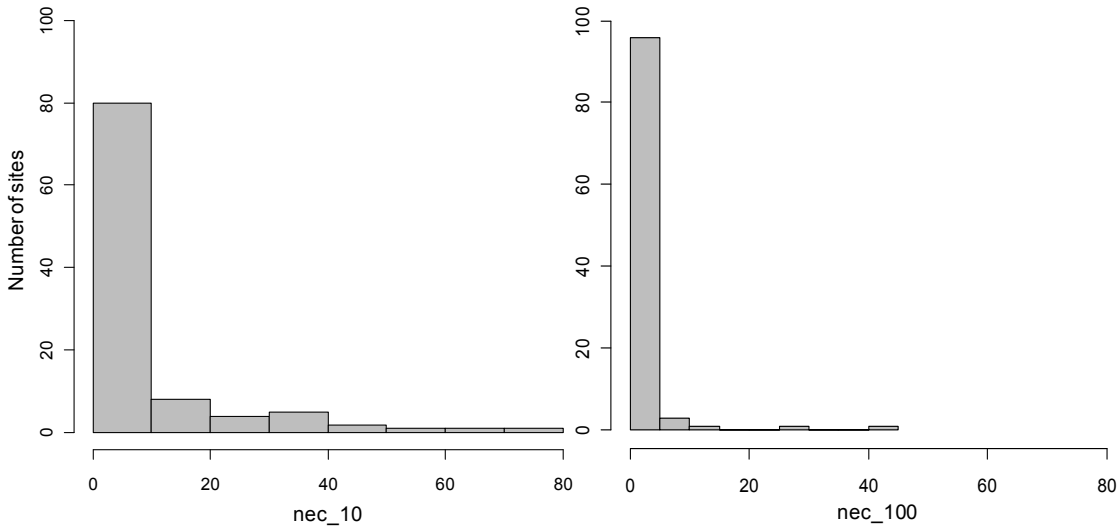


Figure 5. Distribution of the nec_10 and nec_100 variables among surveyed sites.

3.1.2. Mortality pattern among years and depth

The highest median of the nec_10 variable has been observed during summer 1999 and the maximum mortality values appeared in 2003 (year that presented the highest variability). The same patterns were observed for the nec_100 variable (Figs. 6a and 6b, see differences in the Y axis scales). It is worth noting that high percentages of colonies with 100% of necrosis were observed only during 2003.

Mortality impacts in gorgonian populations decreases with depth. Highest levels of mortality were observed at the 10 m depth with important decrease in deeper layers. Although, at 25 m depth great variability was observed, attaining values near 40% of affected colonies at some sites. These high values must be related with the 1999 mortality event that affected deeper populations than the 2003 MME (Perez et al., 2000, Garrabou et al, 2009) (Figs. 6c and 6d).

In general, the response variables show great variability mainly for summer 2003.

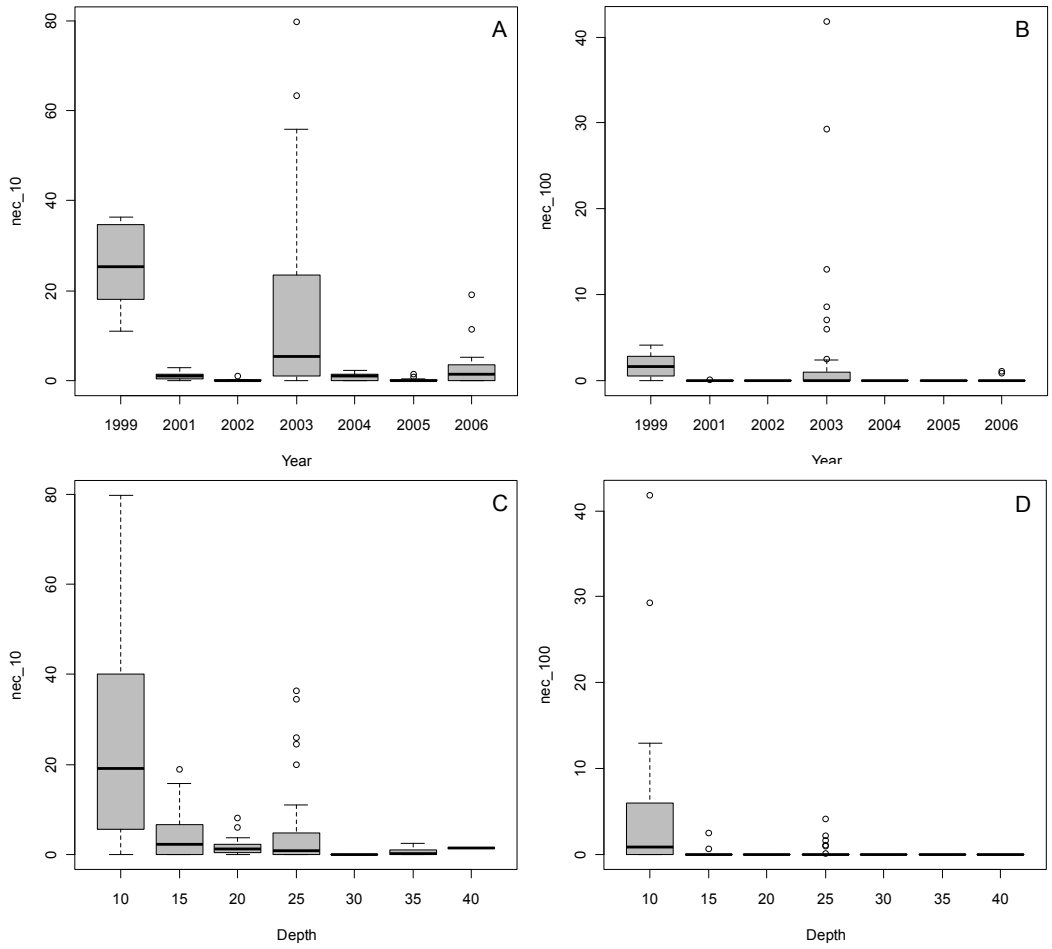


Figure 6. Mortality pattern (nec_10 and nec_100 variables) through years (A and B) and depths (C and D) for both response variables.

3.1.3. Mortality pattern among years within regions

When regarding mortality of different years but within each region separately, inter-regional differences arise. Marseille and Port-Cros attained the highest mortality rates (note the different scales in Y axis) during 1999 (Figs. 7b ans 7c). During 2003, Marseille presented the highest values (Fig. 7b). Medes presented some degree of affectation during this year too, but with lower values (Fig. 7a). In 2006, Marseille and Scandola presented similar values of mortality than those of Medes during 2003 (Figs. 7a, 7b and 7d). We show the results only for the nec_10 variable (Fig. 7).

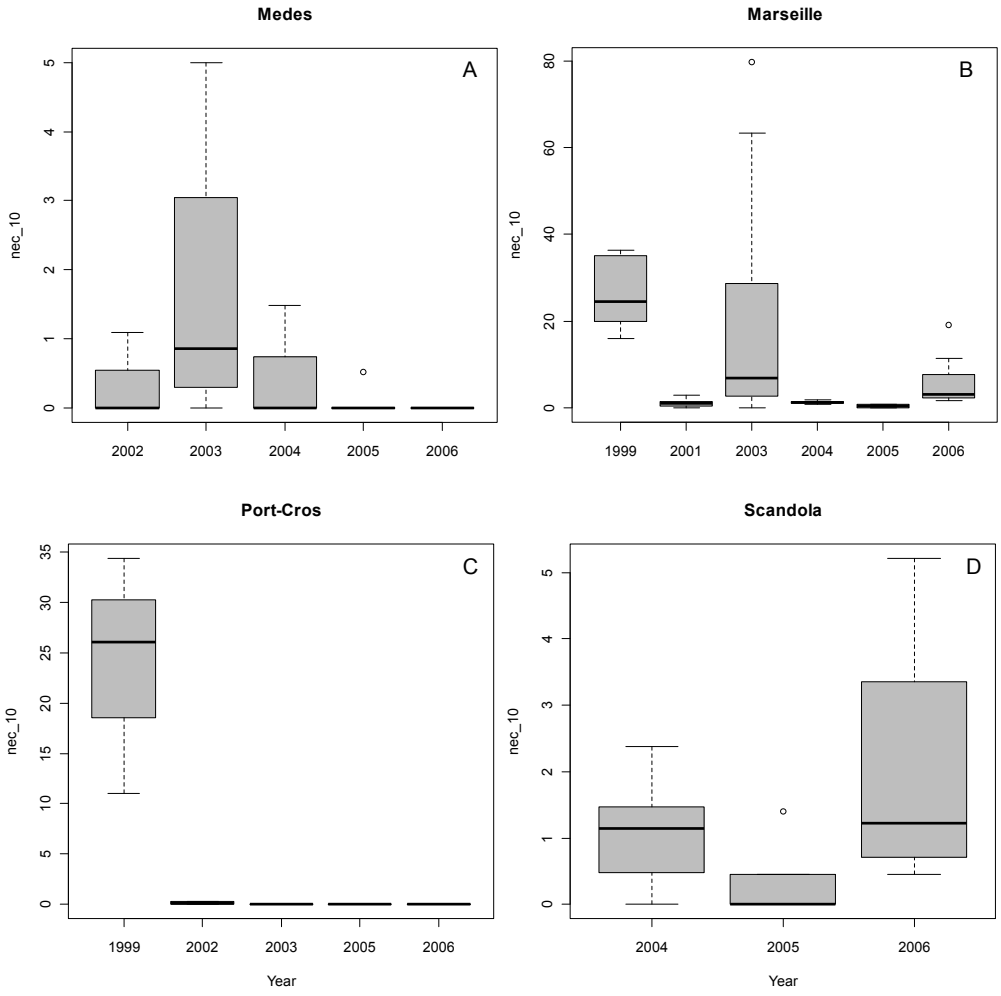


Figure 7. Mortality pattern (nec_10) among years inside each of the studied regions (remark the differences in the ordinates scale).

3.2. Model results

3.2.1. Model errors

The errors of the three models for both response variables and two testing scenarios are reported in Table 3. Performed models presented similar error rates when analyzing the same response variable and testing scenarios (Table 3). Highest error rates were observed when modeling nec_10 response variable with testing scenario #1, indicating that in average, predicted values are 9 to 10% far from the real values. The low error values in the testing scenario #2, both for the nec_10 and the nec_100 variables may be explained by the suitability of any of the three models for predicting mortality form new T data.

Table 3. Model errors for the three selected models with different testing scenarios and for both response variables.

Testing scenario	Dep. Var.	CART	BAGGING	RF
#1	nec_10	10.13	9.26	9.26
	nec_100	3.65	3.62	4.21
#2	nec_10	4.57	5.39	4.68
	nec_100	0.34	0.48	0.86

3.2.2. Regression Trees

Regression trees for the nec_10 and the nec_100 responses are shown on Fig. 8. For the nec_10 response, the MeanT_30 variable (Mean temperature of the 30 consecutive days with highest mean temperature) was sufficient to perform the optimal tree. High mean temperature of relative long periods were associated with the highest mortality rate predicted by the tree (29% of colonies with >=10% of coenenchyme necrosis). Intermediate mortality rates were associated with lower but still high values of MeanT_30. Finally, relative low values of this variable were associated with low mortality rates.

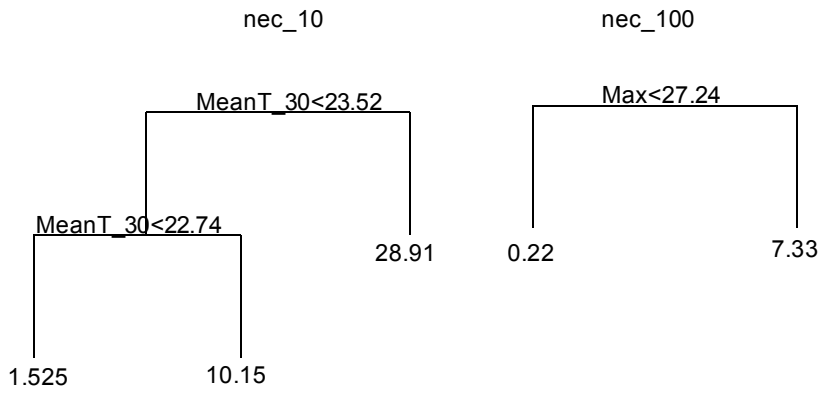


Figure 8. Regression trees representation for the nec_10 and nec_100 variables (testing scenario #1). One variable was enough to explain both nec_10 and nec_100 variability.

For the percentage of affected colonies with 100% of necrosis (nec_100), the Max T was the only variable used to perform the optimal tree. Very high Max T values (over 27°C) were associated to relative high percentage of colonies with 100%.

3.2.3. Random Forest variables importance

Random Forest Variables importance plots (VarImPlot function from the randomForest package) are represented in Figure 9. Retained variables are those that contribute to reduce the MSE in more than 10% (those inside dashed squares). For nec_10, seven variables were retained. Some of them refer to the mean of different length hottest periods (MeanT_2, MeanT_10, MeanT_30, see Table 2), and others to the proportion of the summer at warm temperatures (prop23). Finally, variables related to variability of the thermal regime at different time scales (CV10, CV30 and CV) were also within the most important variables (Fig. 9).

For the nec_100, six variables were retained. As for nec_10, some of them were related to the mean of different length hottest periods (MeanT_2, MeanT_10, MeanT_30).
The other retained variables are the proportion of the summer spent at high temperatures (prop26 and prop27) and the maximum summer temperature (Max) (Fig. 9).

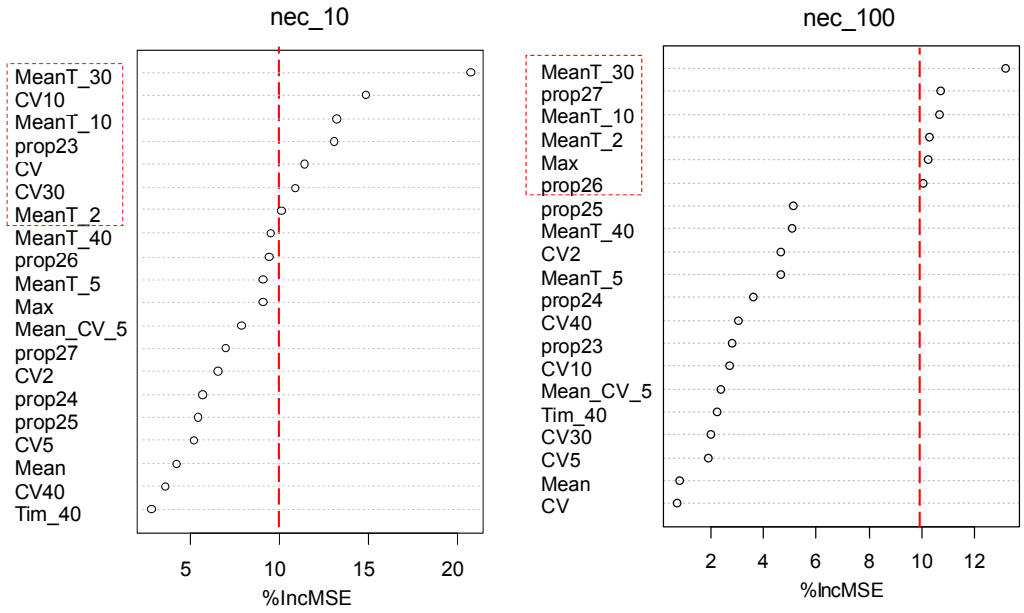


Figure 9. Random Forest variables importance plot for nec_10 and nec_100. The dots represent each predictive variable contribution for decreasing the percentage of Mean Standard Error (MSE) of the model, i.e. the increase of the MSE if the variable is excluded from the model. The retained variables are which contributed in decreasing the MSE of 10% or more (those inside the dashed squares).

3.2.4. Reduced models

Reduced models were performed including only the retained most important variables. For the nec_10 response, seven variables were kept and for the nec_100 six variables were used. The performance of the reduced models for both responses and two testing scenarios were very similar to the ones obtained using all the variables (Table 4).

Table 4. Prediction errors for the reduced models.

Testing scenario	Dep. Var.	CART	BAGGING	RF
#1	nec_10	11.91	10.79	10.62
	nec_100	4.04	4.00	4.05
#2	nec_10	3.26	3.74	3.95
	nec_100	0.34	0.29	0.84

3.2.5. Response variables versus retained predictive variables

To give better insight concerning the used models and their results we analyzed the relations between the responses and the retained variables.

MeanT_2, MeanT_10 and MeanT_30 temperature variables were positively correlated with nec_10 (Figs. 10a, 10b and 10c). Intermediate to high values of MeanT_2 and MeanT_10 were associated to high mortality rates. In the case of MeanT_30, high mortality values were associated only with high values of this variable. The variable prop23 was associated with high mortality rates at relative low values of this variable while high values of this variable were related to lower (but still high) mortality rates (Fig. 10d).

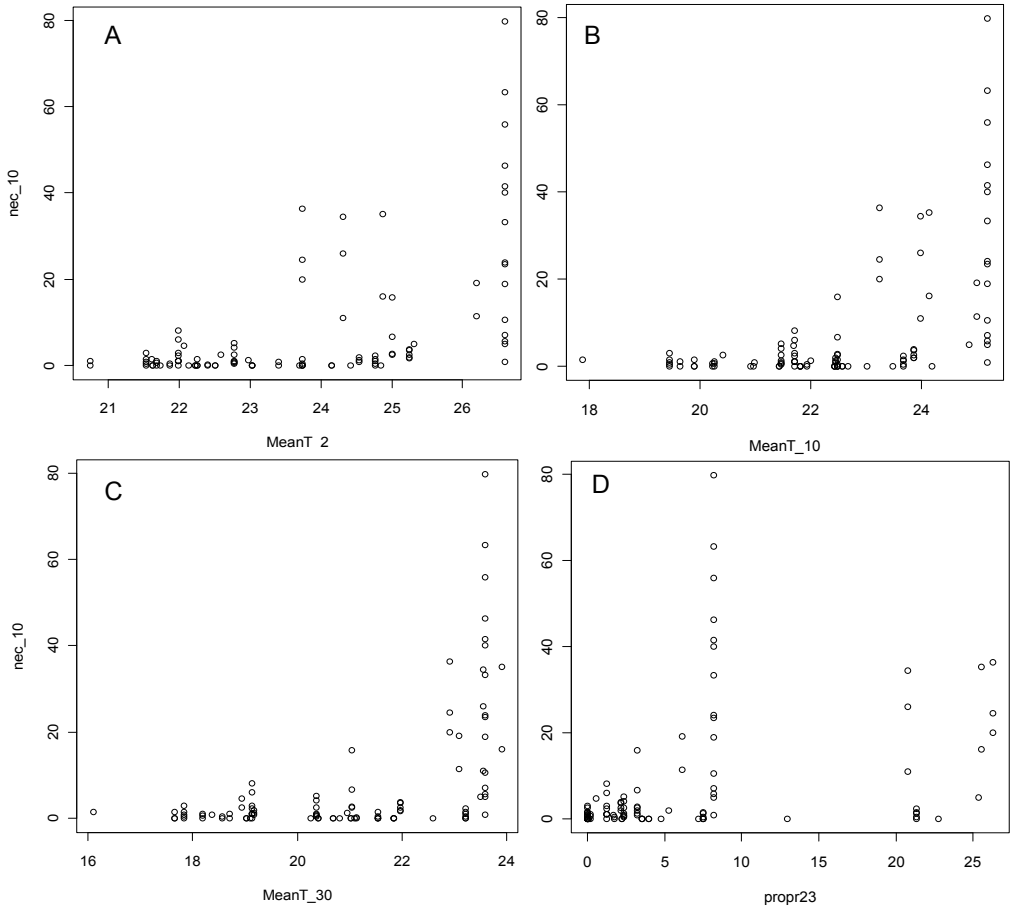


Figure 10. Some of the variables used to model the nec_10 response under a reduced model.

The CV of 10 and 30 consecutive days with the highest mean T were associated with intermediate to high mortality rates at low and intermediate values of the variable. In the

contrary, the CV of the summer period presented intermediate to high mortality rates at high values of the variable (Fig. 11a, 11b and 11c).

In general, predictive variables values associated with high mortality rates were also associated with low and intermediate mortality rates, indicating the great variability in the mortality pattern inside years with MME.

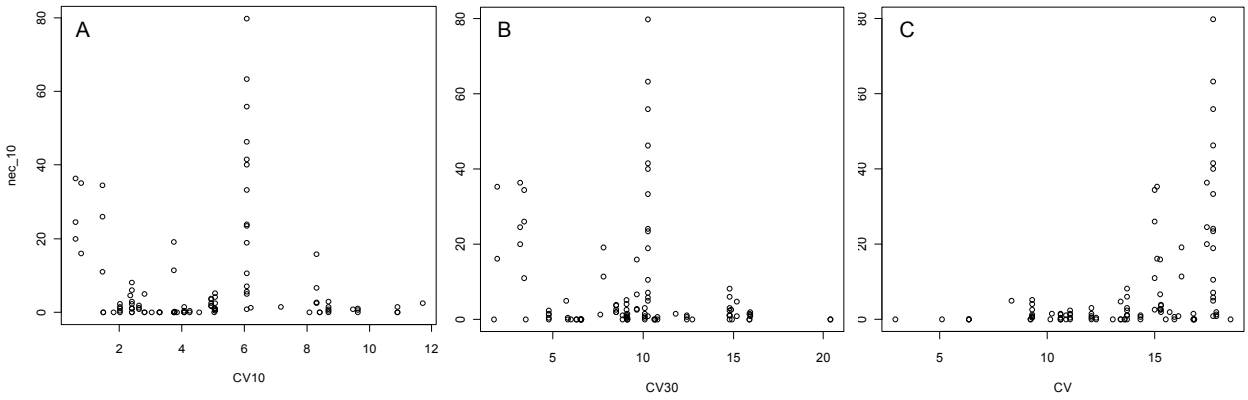


Figure 11. Some of the variables used to model the nec_10 response in the reduced model.

High values of the Max, MeanT_2, MeanT_10, MeanT_30, prop_26 and prop 27 variables were associated with high impacts in the rocky benthic community reflected in high percentage of 100% affected colonies (Figs. 12a to 12f). As for nec_10, the values of these variables associated with high mortality rates were also related to low and intermediate values, accounting for the important variability of the biological response in bearing thermal anomalies.

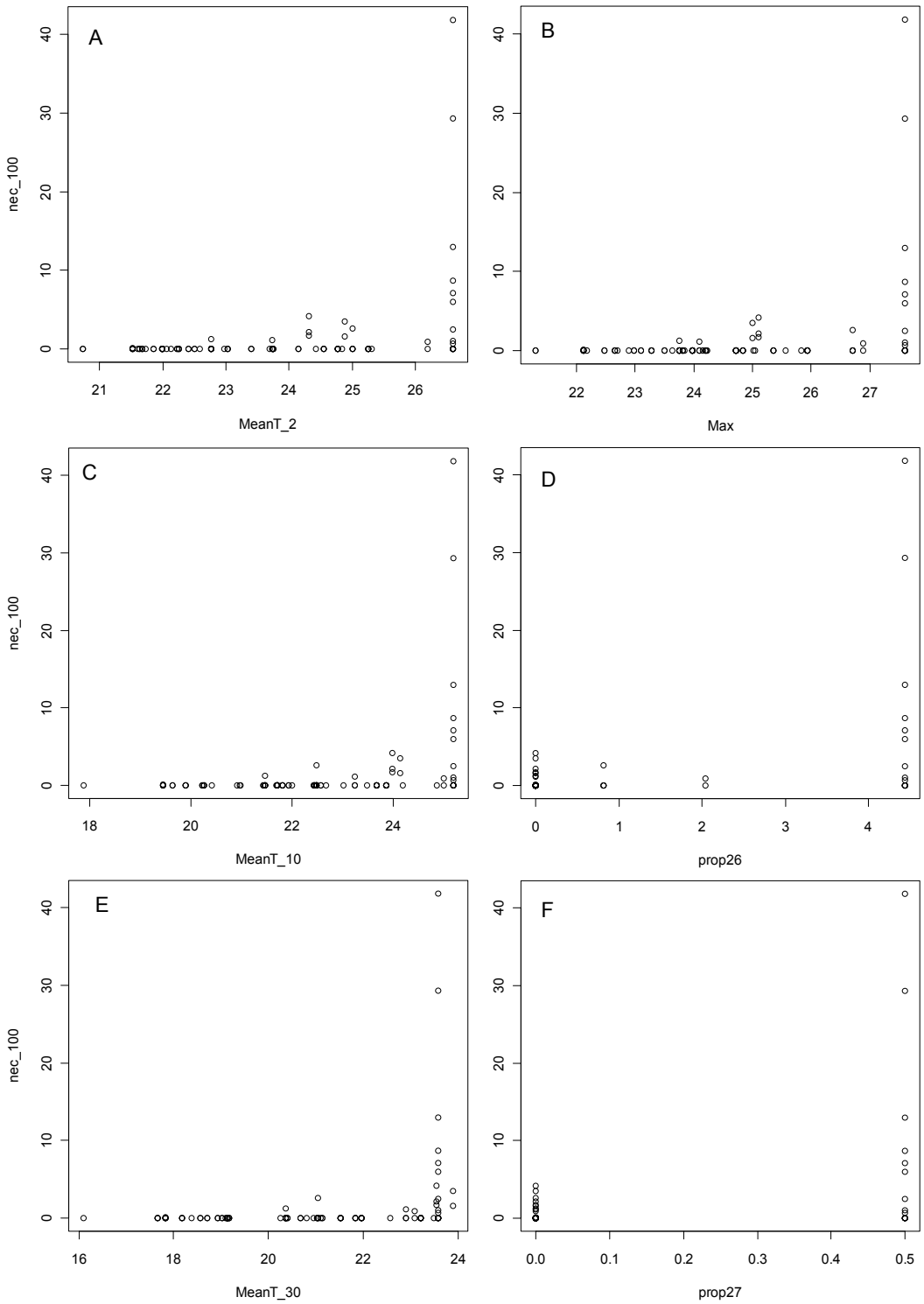


Figure 12. Nec_100 response vs the retained important variables.

In Figure 13 an outline of thermal characteristics associated with different mortality impacts is presented.

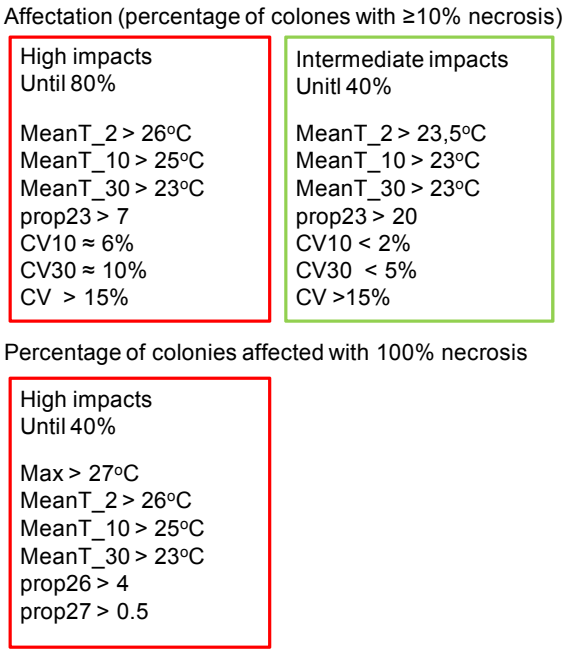


Figure 13. Temperature conditions associated with different mortality impacts in the macrobenthic community. (for the reduced model). Different T situation associated with morality impacts were associated with a great variability of the mortality variables, hence, the level of the impact presented in each square of the figure is the highest level of mortality observed under indicated T conditions. Lower levels of mortality, including null mortality rates were also observed (see Figures 10, 11 and 12).

3.2.6. Predictions with the reduced model

Random Forest mortality predictions (Observed vs Predicted plots) obtained from testing scenarios #1 and #2, under the reduced model are shown in Figure 14. Testing scenario #1 of the nec_10 response, in general gave good predictions of high mortality values, although there were some overestimated and underestimated values. In most of the cases were mortality was underestimated by the model, still high values of mortality were predicted. Most of the cases presented low mortality rates and they were well predicted (Fig. 14a). For the nec_100 variable almost all mortality values were well predicted (most of the values near zero). The highest observed mortality value was predicted with a lower but still high value and only one value was importantly overestimated (Fig. 14b). Finally a relatively high value (~5%) of the variable was underestimated (0%).

When evaluating the model with 2006 mortality data (testing scenario #2), for the nec_10 variable, the highest percentages of affectation observed during 2006 were well predicted. Remaining observed values (between 0 and ~5%) were relatively well predicted since the range of prediction was not far from those of observed values (0 to 8.9%) (Fig. 14c). For the nec_100 variable, very low percentages were observed (0 to 1.2%). Model predictions were in all cases also related to low values (0 to 2.4%) (Fig. 14d).

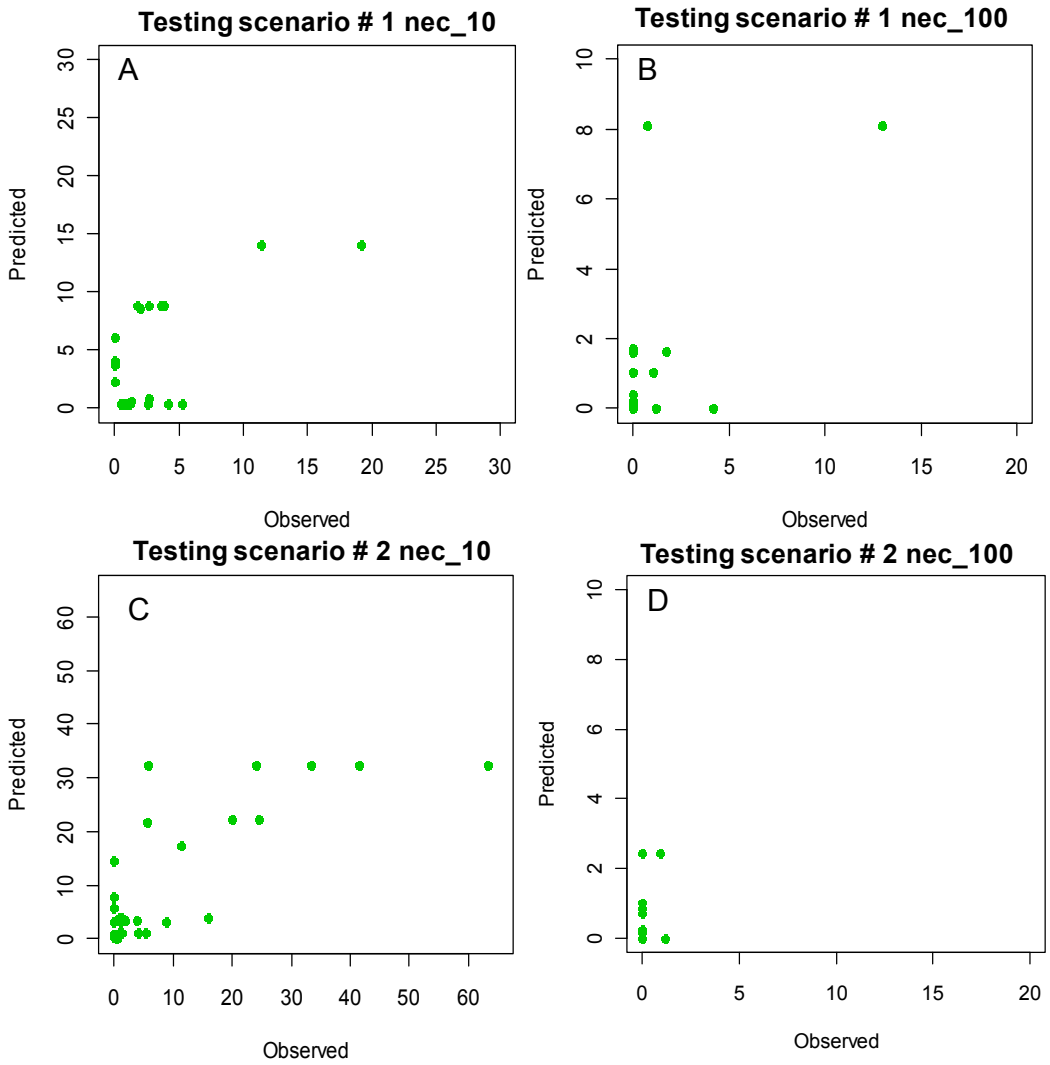


Figure 14. Observed vs predicted mortality for both responses and testing scenarios #1 and #2 (remark the different scale in the ordinates axis).

4. Discussion

In this work we performed Statistical Learning models to provide a predictive tool of future MME in the macrobenthic community of the NWM. In general, different models presented moderate to high performance in predicting mortality. Reduced models using most important variables showed similar performances.

For the nec_10 variable (percentage of colonies affected with 10% or more of tissue necrosis), the models well predicted intermediate to high values of this variable with some over and underestimations. Models overestimation of mortality rates could be explained by the high variability of the biological response observed during the MME (e.g. values from 0 to 80% were registered in Marseille during 2003). Apparently, other factors rather than temperature are acting in conditioning the biological response to temperature since population submitted to the same thermal regime (same region) presented different mortality response. We guess that local adaptation could be behind this differential mortality pattern. In fact, recent studies of the genetic structure of the populations of two of the gorgonian species analyzed in this study (*C. rubrum* y *P. clavata*) identified significant differences in the genetic structure of populations at low spatial scales (Ledoux et al., 2010; Mokhtar-Jamaï et al., 2011a).

As indicated by the plots of retained T variables in the reduced models, against nec_10 variable, high levels of affectation were associated with high Mean T values of short, intermediate and long consecutive periods with the highest mean T and also with high variability in different length periods. Intermediate impacts were associated with lower Mean T values of short and intermediate periods (but still high when comparing with years without mortality), high Mean T values of long periods and great proportion of the summer at warm T. Low variability of short and intermediate periods were also associated with intermediate impacts. The thermal characteristic associated with high levels of mortality impact were very similar to those detected in previous studies for the 2003 and 2006 MME. On the other side, the thermal attributes of intermediate mortality impacts were associated with those found for the 1999 MME (see Chapter 2, Crisci et al., 2011).

Concerning the nec_100 response, very few sites were affected with high levels of this variable. This was expected since this variable reports the percentage of colonies that are totally death. As was previously attested for the 1999 MME, this indicator could be more useful to quantify delayed effects of mortality episodes because of a delayed stress response in the surviving colonies affected by extensive necrosis (Linares et al., 2005). Nevertheless, this variable could be useful to predict immediate effects of mortality events

like that of 2003, probably because of the presence of lethal temperatures that could induce a more immediate effect (see Chapter 3). In fact, among the most remarkable thermal characteristics associated with high impacts of mortality in terms of the percentage of totally (100%) affected colonies were the very high maximum summer T (>27^{o}C) and also the very high temperature of short consecutive periods with the highest mean T.

The modeling nec_100 response gave relative low prediction errors, correctly predicting the low variable values (most of the cases) and the highest variable value. As occurred with the nec_10 response, some mortality overestimation was observed. Again this was probably associated with variable mortality pattern during years with MME.

In general, scatter plots of predictors against response variable show clearly that most relations are not linear at all, and in many cases no transformation of the data seem to be potentially useful to transform relations into linear ones. This is why recursive partition methods as those used here seem to be useful in treating complex structure data. More sophisticated and probably suitable models such as decision trees for zero inflated data (Lee and Jin, 2006), that could better deal with the high number of cases with very low mortality values, are under study. Overall, despite some inaccuracies, the models applied here should be useful to give alerts for future mortality events.

The acquisition of biological (MedRecover, http://www.medrecover.org/) and temperature (t-MedNet,http://www.t-mednet.org) data is of great importance to improve future models predictions. At the same time, modeling future temperature scenarios for different regions of the NWM coastal waters is crucial for using simulated temperature data in the models. Finally, further knowledge of biological aspects of the rocky benthic community, such as the thermotolerance of species or populations (e.g. Torrents et al., 2008, Ferrier-Pages et al., 2009, Chapter 3), genetic features such as mentioned local adaptation (Ledoux et al., 2010; Mokhtar-Jamaï et al., 2011) or genetic diversity (Mokhtar-Jamaï, 2011) could be integrated to the models to increase the accuracy of predictions.

Acknowledgements

We thank C Linares (Universitat de Barcelona) and R Coma (Centre d'Estudis Avançats de Blanes) for providing the biological data from Medes and Port-Cros islands.

Conclusion générale

CHAPITRE 6

Dans ce chapitre, je présente une synthèse des résultats obtenus lors de cette thèse et discute leur importance pour anticiper les effets du changement climatique sur la conservation des communautés benthiques de substrats rocheux en Méditerranée nord-occidentale. Je conclue en proposant quelques perspectives futures de travail.

1. Synthèse des résultats

Cette thèse caractérise précisément le rôle de la température dans les épisodes de mortalité massive observés au cours de ces dernières années au sein des communautés benthiques de substrat rocheux de la Méditerranée nord-occidentale (MNO).

L'étude de séries de température à haute résolution (horaire) a permis une analyse détaillée des caractéristiques thermiques des différentes régions d'étude sur une période de plusieurs années en mettant l'accent sur les deux épisodes de mortalité massive les plus importants jamais observés dans la région (1999 et 2003) ainsi qu'un troisième moins sévère (2006) (Chapitre 2). Ces résultats ont permis d'analyser la relation entre les conditions thermiques et les différences interrégionales et interannuelles des impacts observés dans les populations macrobenthiques (la gorgone rouge *Paramuricea clavata* étant considérée comme une espèce modèle pour cette étude) pendant les épisodes de mortalité massive.

Au cours de la période allant de 1999 à 2006, deux types d'anomalies thermiques ont été détectés. L'une d'elles a été associée à des épisodes de températures extrêmes de courte durée (25-27^{o}C) et l'autre à des épisodes prolongés de températures modérées à élevées (23-24^{o}C). Le moment de l'été où ces épisodes de longue durée se sont produits s'avére déterminant dans l'apparition des mortalités. En effet, une survenue tardive de ces épisodes pendant la période estivale est associée à de forts taux de mortalité tandis qu'une survenue précoce ne provoque pas de mortalité. Au contraire, les épisodes sévères de courte durée induisent systématiquement une mortalité, bien que des taux plus faibles d'affectation aient été observés lorsqu'ils ont lieu en début d'été.

Parallèlement, la variabilité du régime thermique à différentes échelles temporelles est une caractéristique importante pour expliquer les différences observées. Nous avons ainsi détecté une forte variabilité pendant les périodes sévères de courte durée. Par contre, les longues périodes avec des températures modérées à élevées ont présenté une faible variabilité. La figure 1 résume les caractéristiques des différents types d'anomalies et le risque associé d'observer des épisodes de mortalité.

En ce qui concerne la fréquence et les caractéristiques des anomalies mentionnées, nous avons détecté des différences interannuelles et interrégionales. En 1999, nous n'avons observé qu'une anomalie de longue durée dans la région de Marseille (Riou) et dans les eaux Provençales du Parc National de Port-Cros, tandis qu'en 2003 et en 2006 les deux types d'anomalies ont été repérés dans ces régions. En 2003, les sites de Riou et Port-Cros ont été soumis à des anomalies de courte durée, alors qu'une anomalie de longue durée a eu lieu dans les îles Medes à la Catalogne. En 2006, les anomalies ont été de courte durée à Riou et au NO de la Corse (Scandola) et de longue durée dans les îles Medes. Finalement, les épisodes de courte durée ont été limités à des eaux peu profondes (inférieur a 25 m), tandis que ceux de longue durée ont touché des profondeurs plus importantes (25 m).

Il est intéressant de remarquer que les deux vagues de chaleur les plus importantes enregistrées en Europe pendant ces dernières decenies ont provoqué des anomalies thermiques contrastées dans la colonne d'eau dans plusieurs localités de la MNO. Ces différences sont très probablement associées aux caractéristiques hydrologiques des différentes zones d'étude. **Ainsi, l'étude détaillée des conditions thermiques a permis de démontrer que les différences interrégionales et interannuelles des taux de mortalité pendant les EMM sont en partie dues à des différences du régime thermique.**

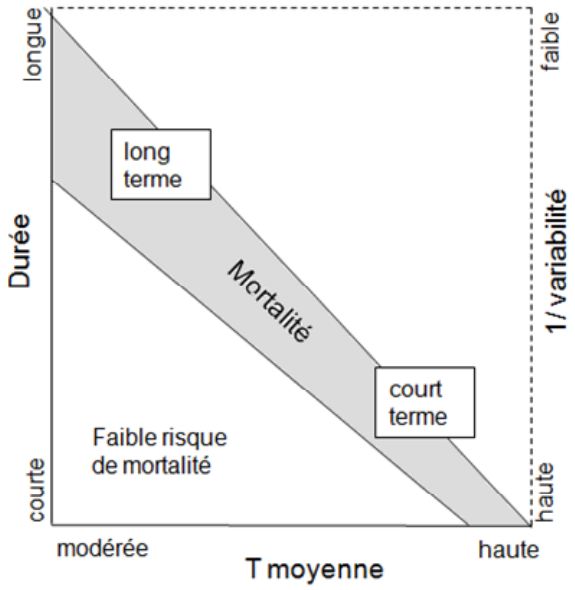


Figure 1. Représentation des caractéristiques thermiques associées aux anomalies thermiques positives et aux épisodes de mortalité massive (en gris) de la communauté benthique de substrats rocheux de la Méditerranée nord-occidentale.

La combinaison des données de température de terrain les plus extrêmes observées pour chaque région avec les expériences de thermotolérance recueillies dans la bibliographie pour différentes espèces d'anthozoaires de la MNO a permis d'identifier des régions avec plus ou moins de risque de subir des épisodes de mortalité massive.

Les conditions de température observées dans les îles Medes se caractérisent par une absence de températures extrêmes (anomalies de courte durée) et par des anomalies de longue durée avec des caractéristiques atténuées. Cette région ne présente pas de conditions thermiques nuisibles pour les organismes pour lesquels nous disposons de données de thermotolérance. Le risque d'épisodes de mortalité dans les îles Mèdes semble donc plus faible que dans les autres régions étudiées. Par contre, Riou et Scandola sont les régions qui ont présenté les anomalies thermiques les plus sévères. Des dépassements des limites de thermotolérance de plusieurs des espèces d'anthozoaires considérées ont en effet été observés dans les deux régions. Le cas de Riou est intéressant car ce site se trouve dans l'une des régions les plus froides de la Méditerranée. Le fait que les deux types d'anomalies puissent se produire, ajouté à la présence à faible profondeur (à partir de 5-10 m) des populations d'espèces affectées, rendent cette région particulièrement vulnérable aux effets des anomalies thermiques. Pendant l'épisode d'anomalie de courte durée de 2006, Scandola a présenté les températures les plus élevées relevées au cours de toutes les années et les régions analysées, ce qui suppose un grand risque pour la communauté macrobenthique sessile de faible profondeur. En fait, les populations de gorgones se trouvent à partir de 20-25m, suggérant que la température est susceptible de d'être un facteur determinant de la distribution en profondeur de ces espèces. Cependant, on ne peut pas écarter d'autres facteurs comme la disponibilité de lumière, l'hydrologie ou la disponibilité en aliments. Finalement, Port-Cros présente une position intermédiaire quant au risque de souffrir de futurs EMM, surtout en raison de l'absence d'épisodes de températures sévères. Il est cependant probable que des épisodes de mortalité soient observés dans cette région suite à des anomalies thermiques de longue durée avec des températures modérées.

Les différences interrégionales et interannuelles du régime thermique ont permis, dans la première partie de la thèse, d'expliquer les différences d'impact observées dans les communautés benthiques. Cependant, étant donné que les populations des différentes régions ont été soumises à différentes magnitudes d'anomalies thermiques, qui se sont produites à différents moments de l'été (c'est-à-dire que les deux types d'anomalies détectés se sont manifestés de manière différente selon les régions et les années), il n'est

pas possible d'analyser l'action d'autres facteurs qui conditionneraient la réponse à la température.

Le fait que les populations de gorgonaires de différentes régions de la MNO soient soumises à des régimes thermiques contrastés combiné à l'importante structuration génétique détectée au sein de certaines espèces, comme la gorgone rouge *P. clavata* et le corail rouge *Corallium rubrum,* même à des échelles spatiales réduites, suggèrent que des facteurs biologiques comme l'acclimatation ou l'adaptation locale pourraient jouer un rôle important dans la réponse des populations à la température.

Afin d'étudier les possibles processus d'acclimatation ou d'adaptation locale au régime thermique, nous avons réalisé des expériences de thermotolérance en considérant des populations provenant de différentes régions de la MNO avec des régimes de température contrastés (chapitre 3). Nous avons analysé et comparé la thermotolérance de populations de *P. clavata* provenant de trois régions de la MNO (îles Medes, Marseille et Scandola), de deux profondeurs (20 et 40 m) et, dans le cas de la région de Marseille, de trois localités à la profondeur de 20 m.

L'échelle spatiale considérée et l'effort expérimental effectué dans ces expériences n'ont pas de précédents pour les espèces méditerranéennes. L'expérience a été développée avec des difficultés et comme tout travail pionnier, malgré les précautions prises, les résultats finaux n'ont pas pu répondre entièrement aux questions initiales.

Les résultats de cette étude ont permis en premier lieu, de connaître la limite de la thermotolérance de l'espèce étudiée. **Ving cinq degrés est une température critique pour *P. clavata* car, indépendamment de leur origine, toutes les populations analysées ont présenté les premiers signes d'affectation à cette température.** Ces résultats coïncident avec ceux trouvés pour d'autres espèces benthiques de Méditerranée telles que le corail rouge (*C. rubrum*) et sont inférieurs à ceux d'autres espèces de gorgonaires comme *Eunicella singularis*. Les températures de 27 et 28^{o}C ont montré d'être létales pour l'espèce.

En deuxième lieu, **la comparaison de la thermotolérance des populations provenant de régions et de profondeurs avec des régimes thermiques contrastés, a mis en évidence que la réponse à la température des différentes populations n'est pas clairement liée au régime thermique des lieux où elles habitent.** Ainsi, **la variabilité entre populations de 20 m de Marseille (même profondeur et même région) s'est révélée plus importante que la variabilité entre régions ou profondeurs, contrairement à ce qui avait été observé lors d'expériences préliminaires.** Nous suggérons donc de

réaliser de nouvelles expériences avec réplication à l'intérieur des régions pour évaluer le niveau de variabilité intrarégionale dans d'autres régions de la MNO.

Les différences rencontrées dans la réponse à la température des populations de Marseille de 20 m pourraient indiquer la présence de processus d'adaptation à des conditions de température locales (plus qu'à des conditions régionales). Comme nous l'avons mentionné ci-dessus, des travaux récents ont prouvé que les populations de *P. clavata* présentent une forte structuration génétique à des échelles spatiales réduites de l'ordre d'une dizaine de mètres, une caractéristique compatible avec des processus d'adaptation locale. Ce résultat a aussi été observé pour d'autres espèces de gorgonaires de la Méditerranée telles que le corail rouge, ainsi que pour d'autres organismes marins (anémones, gastéropodes, bryozoaires) de différentes régions. Il s'avère intéressant que la population de la Grotte Pérès (Marseille) ait montré l'impact le plus faible. Cette population a été exposée à des sources de pollution pendant des décennies (elle est située près de l'émissaire de Cortiou). Elle pourrait donc être mieux adaptée pour supporter d'autres sources de stress que les autres populations de cette même région.

Si l'on superpose les courbes de thermotolérance de *P. clavata* aux conditions de température des différentes régions, on observe clairement que les populations les moins profondes (20 m) vivent près de leur limite de thermotolérance. Au contraire, les populations situées plus en profondeur (40 m) vivent une situation moins critique car les conditions de température sont assez éloignées de la limite de thermotolérance.

Il est important de signaler que bien que la température ait une relation claire avec la mortalité chez différentes espèces de gorgonaires de la Méditerranée, elle agit certainement comme facteur déclenchant ou de manière synergique avec d'autres facteurs comme la présence de micro-organismes pathogènes thermodépendants, l'état physiologique, etc. En effet, les différences rencontrées à différentes échelles spatiales pourraient être associées à un développement différentiel de ces facteurs.

En ce qui concerne l'état physiologique, nous savons, à travers des études préalables, que ce dernier varie de manière saisonnière. Au printemps, les populations de *P. clavata* investissent une grande partie de leur énergie dans le développement des gonades tandis qu'en été, caractérisé par des températures élevées et une faible disponibilité d'aliments, elles subissent un stress physiologique. Étant donné les restrictions énergétiques dont souffrent les suspensivores passifs et, en particulier, les populations de *P. clavata*, nous avons voulu étudier si le changement dans l'état physiologique peut influencer la réponse

des populations face au stress thermique. À cette fin, nous avons comparé la réponse de trois populations peu profondes de Marseille collectées à trois moments de l'année : la fin de l'hiver, la fin du printemps et la fin de l'été.

Contrairement à l'attendu, les tests statistiques n'ont pas montré de différences dans le patron global de mortalité entre les différentes périodes, ce qui indique que l'état physiologique n'influence pas la réponse au stress thermique des populations (probablement que le fait de nourrir les colonies au cours des expériences a contribué à réduire les différences entre les différents expériences).

Néanmoins, l'analyse des différences entre les populations pour chacune de ces trois expériences a montré des différences significatives dans le cas des expériences menées à la fin du printemps et de l'été. Cependant, lorsque les échantillons ont été recueillis à la fin de l'hiver aucune différence n'a été observée.

Ce résultat suggère que lorsque l'état physiologique est faible (fin du printemps et fin de l'été), les populations dépendent de mécanismes comme l'adaptation locale ou l'acclimatation associées aux conditions environnementales locales (comme nous l'avons exposé plus haut pour la population de la Grotte Pérès). Cependant, afin de confirmer cette hypothèse sur l'effet du stress physiologique dans la réponse à la température, des expériences de thermotolérance avec deux types de concentrations d'alimentation (faible et élevée) doivent être envisagées. Ceci permettrait de définir le rôle de l'interaction entre stress thermique et pénurie alimentaire dans le développement de la mortalité pendant la période estivale.

La mer Méditerranée est considérée comme un *hotspot* du changement climatique, puisqu'il devrait y avoir de plus en plus de vagues de chaleur dans le futur. Dans ce scénario, les populations peu profondes de *P. clavata* ainsi que des autres espèces affectées par les EMM en MNO seront exposées à des épisodes récurrents de mortalité massive. Malgré ce scénario pessimiste pour les communautés macrobenthiques sessiles de la MNO, des études empiriques récentes ont démontré l'interaction entre processus écologiques et évolutifs à des échelles de temps réduites et indiquent que des forces sélectives anthropogéniques pourraient promouvoir l'évolution contemporaine (définie comme l'évolution de traits héritables au sein de populations au cours d'une période restreinte de quelques dizaines de générations). Ce processus combiné à la capacité d'acclimatation et à l'adaptation locale pourrait mitiger les impacts potentiels du changement global.

Dans ce sens, une grande partie des populations étudiées lors des expériences ont montré une proportion significative de colonies résistantes (définies comme des colonies avec moins de 30% de nécrose). Ces colonies résistantes pouvant participer à des épisodes de reproduction, leur présence a des implications importantes quant aux capacités d'adaptation ou d'acclimatation des populations de *Paramuricea clavata* dans le contexte des changements environnementaux associés au changement climatique.

Il faut cependant tenir compte de certains traits d'histoire de vie de ces espèces. Les gorgonaires étudiés sont en effet des espèces longévives qui se caractérisent par des dynamiques de populations lentes avec un temps de génération élevé et une capacité de dispersion limitée (limitant la dispersion de génotypes thermorésistants). **Il faut donc considérer dans leur ensemble les caractéristiques écologiques et les processus évolutifs des populations de *P. clavata* ainsi que d'autres espèces de la communauté macrobenthique sessile de la MNO afin de pouvoir évaluer l'effet du changement climatique dans ces communautés.**

Dans le chapitre 4 de cette thèse, nous avons introduit les bases de l'apprentissage statistique et exposé une variété de techniques particulièrement utiles dans le traitement de données écologiques. Dans le chapitre 5, nous avons appliqué certaines de ces techniques pour développer un outil de prédiction des épisodes de mortalité en fonction de la température. Les *Arbres de Régression*, *Bagging* et les *Forêts Aléatoires* ont démontré des taux d'erreur similaires pour chacun des deux variables de mortalité analysées. L'évaluation de ces modèles avec un nouveau jeu de données a été satisfaisante avec de faibles taux d'erreur et une bonne capacité à prédire la mortalité à partir de nouvelles données de température. Les modèles utilisés pour prédire la variable de réponse *nec_10* (pourcentage de colonies avec une nécrose du tissu de plus de 10%) se sont avéré utiles pour prédire différents degrés de mortalité (d'intermédiaires à élevés). D'autre part, la variable *nec_100* (pourcentage de colonies touchées à 100%) s'est avérée utile surtout pour prédire les épisodes les plus sévères.

Les caractéristiques thermiques associées à des niveaux de mortalité intermédiaire ont été très similaires (selon les variables retenues dans un modèle réduit) à celles rapportées dans le Chapitre 2 pour caractériser les événements de mortalité de longue durée, tandis que les variables de température associées à des niveaux de mortalité élevés étaient surtout liées à des caractéristiques rencontrées pour les épisodes de mortalité de courte durée. Il est important de mentionner la grande variabilité rencontrée (depuis des valeurs presque nulles

à de forts niveaux d'affectation) au sein des différents sites au cours des années de mortalité. Par exemple, en 2003 à 10 m de profondeur dans la région de Marseille, nous avons observé des pourcentages de 0 à 80% de colonies touchées à plus de 10%. Cette variabilité a pu provoquer un moins bon calage des modèles. Cependant, **les modèles appliqués se sont révélés utiles pour permettre l'alerte de futurs épisodes de mortalité comme ceux de 1999, 2003 et 2006.** Finalement, l'utilisation de modèles qui ne supposent aucun type de relation entre les variables prédictives et les variables de réponse semblent être plus adéquate pour étudier des problèmes comme ceux de cette thèse, où plusieurs des variables prédictives ont présenté une relation non-linéaire et non-monotone avec la variable réponse (valeurs plus élevées de la variable de réponse à des niveaux intermédiaires de la variable prédictive).

2. Les perspectives

Les résultats obtenus de cette thèse suggèrent l'importance de l'adquisition et de l'analyse des séries de température à haute résolution temporelle et spatiale. La continuité dans l'acquisition de ces données permettra de poursuivre l'analyse des conditions de température responsables des mortalités au sein des communautés benthiques de substrats rocheux de MNO. Il existe actuellement un réseau de données de température T-MedNet (www.t-mednet.org) qui enregistre la température dans 12 localités de MNO avec une haute résolution. Ces séries (qui incluent les séries étudiées dans cette thèse) sont aussi importantes afin d'évaluer les résultats de simulations de température réalisées pour la MNO selon différents scénarios de réchauffement (par exemple en utilisant le modèle MENOR/MARS3D, CLIMCARES Project). La validation de ces simulations et la combinaison des résultats avec des données biologiques obtenues lors d'expériences de thermotolérance permettront de générer des cartes de risque de mortalité dans différentes localités de la MNO. Parallèlement, il semble important d'obtenir de nouvelles données de thermotolérance d'espèces non-étudiées pour générer des cartes de risque plus précises. De même, l'obtention de nouvelles données de température et de mortalité de terrain permettra d'améliorer l'ajustement des modèles statistiques utilisés pour la prédiction d'épisodes de mortalité.

L'approfondissement de la connaissance des réponses biologiques liées aux conditions thermiques et donc à l'apparition de la mortalité, augmentera aussi la précision dans la prédiction d'épisodes futurs et permettra d'en améliorer la compréhension. Par exemple, les études en course sur l'action des micro-organismes pathogènes thermodépendants

associés aux espèces touchées. L'un des aspects qu'il faudrait aussi approfondir pour améliorer la connaissance des épisodes de mortalité est la grande variabilité d'affectation observée à différentes échelles spatiales (au sein d'une même population, entre populations, entre régions). Poursuivre les études génétiques afin d'évaluer les processus d'adaptation locale ou d'acclimatation permettra probablement de définir les causes de la variabilité mentionnée. Finalement, l'effet des températures anormales lors d'étapes précoces du développement (étape larvaire) d'espèces de gorgonaires qui présentent des dynamiques de populations lentes pourrait fournir des pistes à propos de l'impact à long terme d'épisodes de mortalité.

Ainsi, l'obtention, l'analyse et l'intégration de ces informations à travers d'un travail interdisciplinaire sont indispensables pour développer des stratégies de gestion et de conservation afin de tempérer les effets du changement climatique dans ce *hotspot* de biodiversité qu'est la Méditerranée nord-occidentale.

Références bibliographiques

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32-46.

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA for PRIMER: guide to software and statistical methods. PRIMER–E Ltd., Plymouth, United Kingdom, 214 pp.

Antonov J, Levitus S, Boyer TP, Conkright M, O'Brien T et al. (1998) World Ocean Atlas 1998 Vol. 1. In: Temperature of the Atlantic Ocean. NOAA Atlas NESDIS, 27, 166 pp.

Auster P (1998) A conceptual model of the impacts of fishing gear on the integrity of fish habitats. Conservation Biology 12: 1198-1203.

Ballesteros E (2006) Mediterranean coralligenous assemblages: a synthesis of present knowledge. Oceanography and Marine Biology 44: 123-195.

Bally M, Garrabou J (2007) Thermodependent bacterial pathogens and mass mortalities intemperate benthic communities: a new case of emerging disease linked to climate change. Global Change Biology 13: 2078-2088.

Baums IB (2008) A restoration genetics guide for coral reef conservation. Molecular Ecology 17: 2796-2811.

Bavestrello G, Boero F (1986) Necrosi e rigenerazione in Eunicella cavolinii in Mar Ligure. Boll Mus Inst Biol Univ Genova 52: 295-300.

Bavestrello G, Cerrano C, Zanzi D, Cattaneo-Vietti R (1997) Damage by fishing activities in the Gorgonian coral *Paramuricea clavata* in the Ligurian Sea. Aquat Conserv 7: 253-262.

Bell JF (1996) Application of classification trees to habitat preference of upland birds. Journal of Applied Statistics 23: 349–359.

Beniston M (2004) The 2003 heat wave in Europe: A shape of things to come? An analysis based on Swiss climatological data and model simulations, Geophysical Research. Letters 31, L02202, doi:10.1029/2003GL018857, 4.

Benito Garzón M, Blazek R, Neteler M, Sánchez de Dios R, Sainz Ollero H et al. (2006) Predicting habitat suitability with machine learning models: The potential area of *Pinus sylvestris* in the Iberian Peninsula. Ecological Modelling 197: 383–393.

Bensoussan N, Romano JC, Harmelin JG, Crisci C, Pascual J, et al. (2009) Warming trends, regional fingerprints and future trajectories of NW Mediterranean coastal waters. In: UNEP RAC/SPA (eds), Proceedings of the 1st Mediterranean symposium on the conservation of the coralligenous and other calcareous bio-concretions. Tabarka, Tunisia, pp. 167–168.

Bensoussan N, Romano JC, Harmelin JG, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuarine, Coastal and Shelf Science 87: 431-441.

Bethoux JP (1979) Budgets of the Mediterranean Sea - Their dependance on the local climate and on the characteristics of the Atlantic waters. Oceanologica Acta 2: 157–163.

Bethoux JP, Gentili B, Raunet J, Taillez D (1990) Warming trend in the western Mediterranean deep water. Nature 347: 660-662.

Bianchi CN, Morri C (2000) Marine Biodiversity of the Mediterranean Sea: Situation, Problems and Prospects for Future Research. Marine Pollution Bulletin 40: 367-376.

Bianchi CN, Morri C (2003) Global sea warming and “tropicalization” of the Mediterranean Sea: biogeographic and ecological aspects. Biogeographia 24: 319-327.

Bianchi CN (2007) Biodiversity issues for the forthcoming tropical Mediterranean Sea. Hydrobiologia 580: 7-21.

Bindoff NL, Willebrand J, Artale V, Cazenave A, Gregory J, et al. (2007). Observations: Oceanic Climate Change and Sea Level. In: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change [Solomon S, Qinb D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, and. Miller HL (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Boudouresque CF (2004) Marine biodiversity in the Mediterranean: Status of species, populations and communities. Scientific Reports of Port-Cros National Park, France 20: 97–146.

Boury-Esnault N, Perez T, Romano JC, Garrabou J, Chevaldonné P et al. (2006). Evaluation des modifications de la biodiversité marine sous l’influence du changement global en Méditerranée nord-occidentale. In Weber, J. (Ed.) Biodiversité et changement global – Réponses adaptatives au changement global : résultats et prospective. Colloque de restitution IFB – GICC, Paris, 18-20 septembre 2006. IFB, Paris : 58-65.

Boyer T, Conkright ME, Levitus S (1999) Seasonal variability of dissolved oxygen, percent oxygen saturation, and apparent oxygen utilization in the Atlantic and Pacific Oceans. Deep-Sea Research I 46: 1593-1613.

Breiman L, Friedman J, Stone CJ, Olshen RA (1984) Classification and Regression Trees. Wadsworth Internacional Group, Belmont, CA.

Breiman L, (1996a) Bagging predictors. Machine Learning 24:123–140.

Breiman L, (1996b) Heuristic of instability and stabilization in model selection. The Annals of Statistics 24 (6): 2350–2383.

Breiman L (2001) Random forests. Machine Learning 45 (1): 5–32.

Burgeot T, Bocquené G, Porte C, Dimeet J, Santella RM et al. (1996) Bioindicators of pollutant exposure in the northwestern Mediterranean Sea. Marine Ecology Progress Series 131: 125 –141.

Carroll S, Hendry A, Reznick D, Fox C (2007) Evolution on ecological time-scales. Functional Ecology 21: 387-393.

Cebrian E, Uriz MJ, Garrabou J, Ballesteros E (2011) Sponge Mass Mortalities in a Warming Mediterranean Sea: Are Cyanobacteria-Harboring Species Worse Off? PLoS ONE 6(6): e20211.

Cerrano C, Bavestrello G, Bianchi CN, Cattaneo-Vietti R, Bava S, et al. (2000) Catastrophic mass-mortality episode of gorgonias and other organisms in the Ligurian Sea (Northwestern Mediterranean), Summer 1999. Ecology Letters 3: 284:293.

Chaudhuri P, Lo W, Loh W, Yang CC (1995) Generalized regression trees. Statistica Sinica 17 (3): 641–666.

Chevaldonné P, Lejeusne C (2003) Regional warming-induced species shift in north-west Mediterranean marine caves. Ecology Letters 6: 371-379.

Clarke KR and Warwick RM (2001) Changes in marine communities: an approach to statistical analysis and interpretation. 2nd ed. PRIMER-E. Plymouth.

Coles S (1975) A comparison of effects of elevated temperature versus temperature fluctuations on reef corals at Kahe Point, Oahu. Pacific Sciences 29: 15:18.

Coles SL and Brown BE (2003) Coral bleaching capacity for acclimatization and adaptation. Advances in Marine Biology 46:183–223.

Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, et al. (2010) The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLoS ONE 5: e11842.

Coma R, Ribes M, Zabala M, Gili JM (1995) Reproduction and cycle of gonadal development in the Mediterranean gorgonian *Paramuricea clavata*. Marine Ecology Progress Series 117: 173-183.

Coma R, Ribes M, Zabala M, Gili JM (1998) Growth in a modular colonial marine invertebrate. Estuarine, Coastal and Shelf Science 47: 459-470.

Coma R, Ribes M, Gili JM, Zabala M (2000) Seasonality in coastal benthic ecosystems. Trends in Ecology and Evolution 15: 448-453.

Coma R, Linares C, Pola E, Zabala M (2001) Seguiment temporal de la gorgònia *Paramuricea clavata* de les illes Medes. Exercici 2001. In: Zabala M (ed.). Seguiment temporal de l'àrea marina protegida de les illes Medes. Informe anual any 2001. Departament de Medi ambient, Generalitat de Catalunya, Barcelona, Spain.

Coma R, Linares C, Pola E (2003) Seguiment temporal de la gorgònia *Paramuricea clavata* de les illes Medes. Exercici 2003. In: Zabala M (ed.). Seguiment temporal de l'àrea marina protegida de les illes Medes. Informe anual any 2003. Departament de Medi ambient, Generalitat de Catalunya, Barcelona, Spain.

Coma R, Pola E, Ribes M, Zabala M (2004) Long-term assessment of the patterns of mortality of a temperate octocoral in protected and unprotected areas: a contribution to conservation and management needs. Ecological Applications 14: 1466-1478.

Coma R, Ribes M, Serrano E, Jimenez E, Salat J, et al. (2009) Global warming-enhanced stratification and mass mortality events in the Mediterranean. Proceedings of the National Academy of Sciences 106: 6176-6181.

Conover D, Munch S (2002) Sustaining fisheries yields over evolutionary time scales. Science 297: 94-96.

Cossins AR, Bowler K (1987) Temperature biology of animals. Chapman and Hall, London.

Costanza R, Kemp M, Boynton W (1993) Predictability, scale, and biodiversity in coastal and estuarine ecosystems: implications for management. Ambio 22: 88:96.

Crisci C, Bensoussan N, Romano JC, Garrabou J (2011) Temperature Anomalies and Mortality Events in Marine Communities: Insights on Factors behind Differential Mortality Impacts in the NW Mediterranean. PLoS ONE 6(9): e23814. doi:10.1371/journal.pone.0023814

Cutler DR, Edwards TCJ, Beard K, Cutler A, Hess KT, Gibson J, Lawler JJ (2007) Random forest for classification in ecology. Ecology 88 (11): 2783–2792.

Danovaro R, Pusceddu A (2007) Ecomanagement of biodiversity and ecosystem functioning in the Mediterranean Sea: Concerns and strategies. Chemistry and Ecology 23: 347–360

Davison A, Hinkley D (1997) Bootstrap methods and their application. Cambridge Series in Statistical and Probabilistic Mathematics. Cambridge University Press, Cambridge.

Dayton PK, Trush SF, Agardy TM, Hofman, RJ (1995) Environmental effects of marine fishing. Aquatic conservation: Marine and Freshwater ecosystems 5: 205:232.

De'ath G, Fabricius KE (2000) Classification and regression tress: a powerful yet simple technique for ecological data analyses. Ecology 81 (11): 3178–3192.

De'ath G (2007) Boosted trees for ecological modeling and prediction. Ecology 88 (1): 243–251.

Defeo O, Gómez J (2005) Morphodynamics and habitat safety in sandy beaches: life-history adaptations in a supralittoral amphipod. Marine Ecology Progress Series 293: 143–153.

Déqué M, Rowell DP, Lüthi D, Giorgi F, Christensen JH, et al. (2007) An intercomparison of regional climate simulations for Europe: assessing uncertainties in model. Climatic Change 81 (1): 53-70.

Devroye L, Gyorfi L, Lugosi G (1996) A probabilistic theory of Pattern Recognition. Springer-Verlag, New York.

Diffenbaugh N, Pal J, Giorgi F, Gao X (2007) Heat stress intensification in the Mediterranean climate change hotspot. Geophysical Research Letters 34:1–6.

Dreyfus G (2005) Neural networks. Methodology and applications. Springer-Verlag, Berlín.

Duran S, Giribet G, Turon X (2004) Phylogeographical history of the sponge *Crambe crambe* (porifera, poecilosclerida): range expansion and recent invasion of the Macaronesian islands from the Mediterranean Sea. Molecular Ecology 13: 109:122.

Efron B, Tibshirani RJ (1994). An Introduction to the Bootstrap. Chapman & Hall, New York.

Efron B, Hastie T, Johnstone I, Tibshirani R (2004). Least angle regression. Annals of Statistics 32: 407- 499.

Fernandes J, Irigoien X, Goikoetxea N, Lozano JA, Inza I, et al. (2010) Fish recruitment prediction, using robust supervised classification methods. Ecological Modelling 221: 338–352.

Ferrière R, Dieckmann U, Couvet D (2004) Evolutionary Conservation Biology. Cambridge University Press, Cambridge.

Ferrier-Pages C, Tambutte E, Zamoum T, Segonds N, Merle PL et al. (2009) Physiological response of the symbiotic gorgonian Eunicella singularis to a long-term temperature increase. Journal of Experimental Biology 212: 3007-3015.

Fielding A (1999) Machine Learning Methods for Ecological Applications. Kluwer Academic Publishers, Massachusetts.

Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20:51–65.

Flach P (2001) On the state of the art in machine learning: a personal review. Artificial Intelligence 13 (1): 199–222.

Francour P, Boudouresque CF, Harmelin-Vivien M, Harmelin JG, Quignard JP (1994) Are the Mediterranean waters becoming warmer? Information from biological indicators. Marine Pollution Bulletin 28: 523-526.

Fredj G, Bellan-Santini D, Meinardi M (1992) État des connaissances sur la faune marine Méditerranéenne. Bulletin Institute Oceanographique Monaco 9: 133–145.

Freund Y (1995) Boosting a weak learning algorithm by majority. Information and Computation 121 (2): 256–285.

Freund Y, Schapire RE (1995) A decision-theoretic generalization of online learning and an application to boosting. In: European Conference on Computational Learning Theory. pp. 23–37.

Friedman JH, Stuetzle W (1981) Projection pursuit regression. Journal of the American Statistical Association 76 (376): 817–823.

García-Castellanos D, Estrada F, Jiménez-Munt I, Gorini C, Fernández M et al. (2009) Catastrophic flood of the Mediterranean after the Messiniansalinity crisis. Nature 462: doi:10.1038/nature 08555.

Garrabou J, Perez T, Sartoretto S, Harmelin JG (2001) Mass mortality event in red coral (*Corallium rubrum*, Cnidaria, Anthozoa, Octocorallia) population in the Provence region (France, NW Mediterranean). Marine Ecology Progress Series 217: 263-272.

Garrabou J, Ballesteros E, Zabala M (2002) Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuarine, Coastal and Shelf Science 55: 493-508.

Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonné P et al. (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global Change Biology 15: 1090:1103.

Gattuso JP, Frankignoulle M, Wollast R (1998) Carbon and carbonate metabolism in coastal aquatic ecosystems. Annual Review of Ecology, Evolution, and Systematics, 29: 405-434.

Gili JM, Ballesteros E (1991) Structure of cnidarian populations in Mediterranean sublitoral benthic communities as a result of adaptation to different environmental conditions. Oecologia Aquatica 10: 243-254.

Gili JM, Coma R (1998) Benthic suspension feeders: their paramount role in littoral marine food webs. Trends in Ecology and Evolution 13: 316-321.

Giuliani S, Lamberti CV, Sonni C, Pellegrini D (2005) Mucilage impact on gorgonians in the Tyrrhenian sea. Science of the Total Environment 353: 340-349.

Goreau T (1959) The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions. Biological Bulletin 115: 59-75.

Goreau TJ, Hayes RL, Strong AE (1997) 1989-1996 Caribbean sea surface temperatures and coral bleaching. Proc. AMLC 28: 44.

Grant PR, Grant BR (2002) Unpredictable evolution in a 30-year study of Darwin's finches. Science 296:707-711.

Grubb T, Bowerman W (1997) Variations in breeding bald eagle responses to jets, light planes and helicopters. Journal of Raptor Research 31: 213–222.

Guisan A, Zimmermann NE (2000) Predictive habitat distribution models in ecology. Ecological Modelling 135 (1): 147–186.

Guisan A, Edwards TC, Hastie T (2002) Generalized linear and generalized additive models in studies of species distributions: setting the scene. Ecological Modelling 157 (2): 89–100.

Guyon I, Elisseff A (2003) An introduction to variable and feature selection. Journal of Machine Learning Research 3: 1157–1182.

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F et al. (2008) A Global Map of Human Impact on Marine Ecosystems. Science 319: 948-952.

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1).

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB et al. (2006) The impacts of climate change in coastal marine systems. Ecology Letters 9: 228-241.

Harmelin JG (1984) Biologie du Corail Rouge Paramètres de populations, croissance et mortalité. Etat des connaissances en France. In: Charbonnier D, Garcia S (eds.). Rapport de consultation technique du CGPM sur les ressources du corail rouge de la Méditerranée occidentale et leur exploitation rationnelle. FAO rapport N° 306 sur les Pêches, Palma de Mallorca, pp 99-103.

Harmelin JG, Marinopoulos J (1994) Population structure and partial mortality of the gorgonian *Paramuricea clavata* (Risso) in the North-Western Mediterranean (France, Port-Cros Island). Marine Life 4: 5-13.

Harmelin JG (2004) Environnement thermique du benthos côtier de l'île de Port-Cros (parc national, France, Méditerranée nord-occidentale) et implications biogéographiques. Scientific Reports Port-Cros national Park, Fr 20: 173:194.

Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR et al. (1999) Emerging marine diseases - Climate links and anthropogenic factors. Science 285: 1505-1510.

Harvell C, Mitchell CE, Ward JR, Altizer S, Dobson A, Ostfeld R, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296: 2158- 2162.

Hastie T, Tibshirani R (1990). Generalized Additive Models. Chapman & Hall, London.

Hastie T, Tibshirani R, Friedman JH (2003) The Elements of Statistical Learning. Springer, New York.

Hastie T, Rosset S, Tibshirani R, Zhu J (2004) The entire regularization path for the support vector machine. Journal of Machine Learning Research 5: 1391–1415.

Hendry AP, Kinnison MT (1999) The pace of modern life: measuring rates of contemporary microevolution. Evolution 53: 1637:1653.

Hendry AP, Kinnison, MT (1999) The pace of modern life: measuring rates of contemporarymicroevolution. Evolution 53: 1637-1653.

Hendry AP, Lohmann LG, Conti E, et al. (2010) Evolutionary biology in biodiversity science, conservation and policy: a call to action. Evolution 64: 1517-1528.

Hoegh-Guldberg O, Smith GJ (1989) The effect of sudden changes in temperature, light and salinity on the population density and export of zooxanthellae from the reef corals *Stylophora pistillata* Esper. and *Seriatopora hystrix* Dana. Journal of Experimental Marine Biology and Ecology 129: 279-303.

Hopkins TS (1985) Physics of the sea. In: Margalef R (ed.). Key environments: Western Mediterranean. Pergamon Press, New York.

Maldonado A (1985) Evolution of the Mediterranean basins and a detailed reconstruction of the Cenozoic paleoceanography. In: Margalef R (ed.) Key Environments: Western Mediterranean. Pergamon Press, Oxford.

Huete-Stauffer C, Vielmini I, Palma M, Navone A, Panzalis P, et al. (2011) *Paramuricea clavata* (Anthozoa, Octocorallia) loss in the Marine Protected Area of Tavolara (Sardinia, Italy) due to a mass mortality event. Marine Ecology 32: 107-116.

Hughes D (1992) Genotype-environment interactions and relative clonal fitness in a marine bryozoan. Journal of Animal Ecology 61: 291-306.

Hughes R and Taylor M (1997) Genotype-environment interaction expressed in the foraging behavior of dogwhelks, *Nucella lapillus* (L), under simulated environmental hazard. Proceedings of the Royal Society of London Series B-Biological Sciences 264: 417-422.

Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR et al. (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301: 929-933.

Hughes TP, Bellwood DR, Folke C, Steneck RS, Wilson J (2005) New paradigms for supporting the resilience of marine ecosystems. Trends in Ecology and Evolution 20: 380:386.

Huttmann F, Lock A (1997) A new software system for the pirop database: data flow and an approach for a seabird-depth analysis. ICES Journal of Marine Science 54 (4):518–523.

IPCC (2007) Climate change 2007: the physical science basis. Contribution of working group I to the Fourth Assessment Report of the intergovernmental panel on climate change (eds. Solomon SD, Qin M, Manning Z, et al.), 996pp. Cambridge University Press, Cambridge and New York.

Jackson J, Kirby M, Berger W, Bjorndal KA, Botsford LW, et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293: 629:638.

Johannesson K, Johannesson B, Varez-Rolanal E (1993) Morphological-differentiation and genetic cohesiveness over a microenvironmental gradient in the marine snail *Littorina saxatilis*. Evolution 47:1770-1787.

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs 8:155–162.

Jokiel PL. (2004).Temperature stress and coral bleaching. In E Rosenberg, Y Loya, (eds.) Coral health and disease. Springer, Berlin. pp 401:425.

Kappel CV (2005) Losing pieces of the puzzle: threats to marine, estuarine, and diadromous species. Frontiers in Ecology and the Environment 3: 275:282.

Karl TR, Trenberth KE (2003) Modern Global Climate Change. Science 302: 1719-1723.

Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters 7:1225-1241.

Kersting DK, Linares C (2009) Mass mortalities of *Cladocora caespitosa* in relation to water temperature in the Columbretes Islands (NW Mediterranean). ASLO Aquatic Sciences Meeting 2009. Nice, France.

Knudby A, Brenning A, LeDrew E (2010) New approaches to modeling fish habitat relationships. Ecological Modelling 221:503–511.

Kohavi R, John GH (1997) Wrappers for feature subset selection. Artificial Intelligence 97 (1-2): 273–324.

Koslow J (2007) The silent deep: The discovery, ecology, and conservation of the deep sea. Univ. of Chicago Press, Chicago.

La Rivière M, Garrabou J, Bally M (2010). Spatial and temporal analysis of microbial diversity associated with the Mediterranean gorgonian *Paramuricea clavata*. 13th International Symposium on Microbial Ecology, Seattle, USA.

Lasker HR (1991) Population growth of a gorgonian coral: equilibrium and non-equilibrium sensitivity to changes in life history variables. Oecologia 86: 503-509.

Ledoux JB (2010) Biologie de la conservation du corail rouge, *Corallium rubrum* (Linnaeus, 1758): Impact du changement global sur l'évolution des populations infralittorales en Méditerranée Nord-Occidentale. PhD Thesis (Université de la Méditerranée, Marseille, France).

Ledoux JB, Mokhtar-JamaÏ K, Roby C, Feral JP, Garrabou J et al. (2010a) Genetic survey of shallow populations of the Mediterranean red coral [*Corallium rubrum* (Linnaeus, 1758)]: new insights into

evolutionary processes shaping nuclear diversity and implications for conservation. Molecular Ecology 19: 675-690.

Ledoux JB, Garrabou J, Bianchimani O, Drap P, Feral JP et al. (2010b) Fine-scale genetic structure and inferences on population biology in the threatened Mediterranean red coral, *Corallium rubrum*. Molecular Ecology 19:4204–4216.

Lee SK, J S (2006) Decision tree approaches for zero-inflated count data. Journal of Applied Statistics 33 (8): 853-865.

Lesser MP (1997) Oxidative stress causes coral bleaching during exposure to elevated temperatures. Coral Reefs 16: 187–192.

Lesser MP, Stochaj WR, Tapley DW, Shick JM (1990) Bleaching in coral reef anthozoans: effects of irradiance, ultraviolet radiation and temperature on the activities of protective enzymes against active oxygen. Coral Reefs 8:225–232.

Levitus S, Antonov JI, Boyer TP (2005) Warming of the World Ocean, 1955-2003. Geophysical Research Letters, 32, L02604, doi:10.1029/2004GL021592.

Linares C, Coma R, Diaz D, Zabala M, Hereu B ey al. (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Marine Ecology Progress Series 305: 127-137.

Linares C (2006) Population Ecology and Conservation of a long-lived marine species: the red gorgonian *Paramuricea clavata*. PhD Thesis (Universitat de Barcelona, Barcelona, Spain).

Linares C, Coma R, Mariani S, Díaz D, Hereu B et al. (2008) Early life history of the Mediterranean gorgonian Paramuricea clavata: implications for population dynamics. Invertebrate Biology 127, 1-11.

Linares C, Doak D (2010a) Forecasting the combined effects of disparate disturbances on the persistence of long-lived gorgonians: the case study of *Paramuricea clavata*. Marine Ecology Progress Series 402: 59–68.

Linares C, Bianchimani O , Torrents O, Marschal, C, Drap P, Garrabou J (2010b) Marine Protected Areas and the conservation of long-lived marine invertebrates: the Mediterranean red coral. Marine Ecology Progress Series 402: 69–79.

Loh W, Vanichsetakul N (1988) Tree-structured classification via generalized discriminant analysis. Journal of the American Statistical Association 83 (403): 715–728.

Lotze H, Lenihan H, Bourque B, et al. (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312, 1806-1809.

Margalef R (1985) The Western Mediterranean. Pergamon Press, London.

McArdle BH, Anderson, MJ (2001) Fitting multivariate models to community data: a comment on distance based redundancy analysis. Ecology 82: 290-297.

McClanahan TR, Ateweberham M, Muhando A, Maina J, Mohammed M.S (2007) Effects of climate and seawater temperature variation on coral bleaching and mortality. Ecological Monographs 77(4): 503-525.

Millot C (1979) Wind induced upwellings in the Gulf of Lions. Oceanologica Acta 2: 261-274.

Millot C (1990) La circulation générale en Méditerranée occidentale. Annales de Géographie 549: 497-515.

Mistri M, Ceccherelli VU (1996) Effects of a mucilage event on the Mediterranean gorgonian Paramuricea clavata. I-Short term impacts at the population and colony levels. Italian Journal of Zoology 63: 221-230.

Mokhtar-Jamaï K (2011) Biologie de la conservation de la gorgone rouge de Méditerranée, *Paramuricea clavata*, dans le contexte actuel du changement climatique. PhD Thesis (Université de la Méditerranée, Marseille, France).

Mokhtar-Jamaï K, Pascual M, Ledoux JB, Coma R, Feral JP et al (2011) From global to local genetic structuring in the red gorgonian *Paramuricea clavata*: the interplay between oceanographic conditions and limited larval dispersal. Molecular Ecology 20: 3291-3305.

Morris CW, Autret A, Boddy L (2001) Support vector machines for identifying organisms, a comparison with strongly partitioned radial basis function networks. Ecological Modelling 146: 57–67.

Myers N, Mittermeier RA, Mittermeier CG, da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403: 853–858.

Nelder J, Wedderburn R (1972) Generalized linear models. Journal of the Royal Statistical Society 135 (3): 370–384.

Nerini D, Ghattas B (2007) Classifying densities using functional regression trees: Applications in oceanology. Computational Statistics & Data Analysis 51 (10): 4984–4993.

Nicholls RJ, Wong PP, Burkett VR, Codignotto JO, Hay JE et al. (2007) Coastal systems and low-lying areas. In: ML Parry, OF Canziani, JP Palutikof, PJ van der Linden and CE Hanson (eds.). Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press. pp. 315:356.

NOAA, National Oceanic and Atmospheric Administration (2009) Large marine ecosystems Web page: http://www.lme.noaa.gov/. The Mediterranean LME. Accessed December, 2009.

Oliver JK, Berkelmans R, Eakin CM (2009) Coral bleaching in space and time. In: van Oppen M, Lough JM (eds.) Coral bleaching: Patterns, processes, causes and consequences. Springer, Heidelberg, pp 21–39.

Oliver TA and Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs DOI 10.1007/s00338-011-0721-y.

Park M, Hastie T (2006) L1 Regularization Path Algorithm for Generalized Linear Models. Technical report, Standford University, Stanford.

Pérez T, Garrabou J, Sartoretto S, Harmelin JG, Francour P, et al. (2000) Mortalité massive d'invertébrés marins: un évènement sans précédent en Méditerranée nord-occidentale. Comptes Rendus de l´Académie des Sciences Paris, Sciences de la Vie 323: 853-865.

Petrenko AA (2003) Circulation features in the Gulf of Lions, NWMediterranean Sea; importance of inertial currents. Oceanologica Acta 26: 323–338.

Pey A, Zamoum T, Allemand D, Furla P, Merle PL (2011) Depth-dependant thermotolerance of the symbiotic Mediterranean gorgonian *Eunicella singular*is: Evidence from cellular stress markers. Journal of Experimental Marine Biology and Ecology 404(1-2) 73-78.

Phetkaew T, Kijsirikul B, Rivepiboon W (2003) Multiclass classification of support vector machines by reordering adaptive directed acyclic graph. International Workshop on Intelligent Systems.

Pinardi N, Arneri E, Crise A, Ravaioli M, Zavatarelli M (2006) The physical, sedimentary and ecological structure and variability of shelf areas in the Mediterranean sea (27). In: Robinson AR, Brink KA (eds.). The Sea Harvard University Press pp 1245-1331.

Prada C, Schizas N, Yoshioka P (2008) Phenotypic plasticity or speciation? A case from a clonal marine organism. BMC Evolutionary biology 8.

Previati M, Scinto A, Cerrano C, Osinga R (2010) Oxygen consumption in Mediterranean octocorals under different temperatures. Journal of Experimental Marine Biology and Ecology 390: 39-48.

Rahmstorf, S (2003) Thermohaline circulation: The current climate. Nature 421, 699.

Ramsay J, Silverman B (2002) Applied functional data analysis: methods and case studies. Springer series in statistics. Springer, New York.

Rebetez M, Dupont O, Giroud M (2009) An analysis of the July 2006 heatwave extent in Europe compared to the record year of 2003. Theoretical and Applied Climatology 95: 1-7.

Ribeiro R, Torgo L, (2008) A comparative study on predicting algae blooms in Douro river, Portugal. Ecological Modelling 212: 86–91.

Ribes M, Coma R, Gili JM (1999) Heterogeneous feeding in benthic suspension feeders: the natural diet and grazing rate of the temperate gorgonian Paramuricea clavata (Cnidaria:Octocorallia) over a year cycle. Marine Ecology Progress Series 183: 125-137.

Ribic C, Ainley D (1997) The relationships of seabird assemblages to physical habitat features in pacific equatorial waters during spring 1984-1991. ICES Journal of Marine Science 54 (4): 593–599.

Riegl B, Piller WE (2003) Possible refugia for reefs in times of environmental stress. International Journal of Earth Science 92:520–531.

Rodolfo-Metalpa R, Bianchi CN, Peirano A, Morri C (2005) Tissue necrosis and mortality of the temperate coral *Cladocora caespitosa* (L., 1767). Italian Journal of Zoology 72: 271–276.

Rodolfo-Metalpa R, Richard C, Allemand D, Ferrier-Pages C (2006a) Growth and photosynthesis of two Mediterranean corals, *Cladocora caespitosa* and *Oculina patagonica*, under normal and elevated temperatures. Journal of Experimental Biology 209: 4546-4556.

Rodolfo-Metalpa R, Richard C, Allemand D, Bianchi CN, Morri C, Ferrier-Pagès C (2006b) Response of zooxanthellae in symbiosis with the Mediterranean corals Cladocora *caespitosa* and *Oculina patagonica* to elevated temperatures. Marine Biology 150: 45-55.

Rodolfo-Metalpa R, Peirano A, Houlbrèque F, Abbate M, Ferrier-Pagès C (2008). Effects of temperature, light and heterotrophy on the growth rate and budding of the temperate coral *Cladocora caespitosa*. Coral Reefs 27 : 17-25.

Rogan J, Franklin J, Stow D, Miller J, Woodcock C, Roberts D (2008) Mapping land-cover modifications over large areas: A comparison of machine learning algorithms. Remote Sensing of Environment 112: 2272–2283.

Romano JC, Bensoussan N, Younes WAN, Arlhac D (2000) Anomalie thermique dans les eaux du golfe de Marseille durant l'été 1999. Une explication partielle de la mortalité massive d'invertébrés fixés ? CR Acad Sci Paris III 323: 415-427.

Romano J, Lugrezi M (2007) Série du marégraphe de Marseille: mesures de températures de surface de la mer de 1885 à 1967. Comptes Rendus Geosciences 339: 57-64.

Romano JC, Lugrezi MC, Durand D, Durand-Le-Breton F (2010) Série du marégraphe de Marseille mesures de températures de surface de la mer de 1895 à 1956 : une correction. Comptes Rendus Geosciences 324 (12): 873-880.

Rosenblatt F (1962) Principles of Neurodynamics: Perceptron and Theory of Brain Mechanisms. Spartan Books, Washington DC.

Rossi S (2002) Environmental factors affecting the trophic ecology of benthic suspension feeders. PhD Thesis (Universitat de Barcelona, Barcelona, Spain).

Ryder TJ, Irwin L (1987) Winter habitat relationships of pronghorns in southcentral wyoming. The Journal of wildlife management 51 (1): 79–85.

Salat J, Pascual J (2002) The oceanographic and meteorological station at L'Estartit (NW Mediterranean). CIESM Workshop Series, 22-24 avril, Monaco pp. 134.

Salomon S, Qin D, Manning M, Maquis M, Averys K et al. (2007) Intergovernmental Panel Climate Change. Fourth's assessment report: The Physical Science Basis. Geneve.

Sammarco PW, Winter A, Stewart JC (2006) Coefficient of variation of sea surface temperature (SST) as an indicator of coral bleaching. Marine Biology 149: 1337:1344.

Schär C, Vidale PL, Lüthi D, Frei C, Häberli C, et. al. (2004) The role of increasing temperature variability in European summer heat waves. Nature 427: 332:336.

Schmidt-Nielsen K (1996) Animal physiology: adaptation and environment. Cambridge University Press, Cambridge.

Segal MR (1992) Tree-Structured Methods for Longitudinal Data. Journal of the American Statistical Association 87 (418): 407–418.

Shan Y, Paull D, McKay R (2006) Machine learning of poorly predictable ecological data. Ecological Modelling 195: 129–138.

Sherman CDH, Ayre DJ (2008) Fine-scale adaptation in a clonal sea anemone. Evolution 63: 1373-1380.

Skelly D, Joseph L, Possingham H, *et al.* (2007) Evolutionary responses to climate change. Conservation Biology 21: 1553-1555.

Smith-Keune C, Oppen M (2006) Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef. Coral Reefs 25: 493-502.

Somot S, Sevault F, Déqué M, Crépon M (2008) 21st century climate change scenario for the Mediterranean using a coupled atmosphere-ocean regional climate model. Global and Planetary Change 63 (2-3) 112: 126.

Sparnocchia S, Schiano ME, Picco P, Bozzano R., Cappelletti A (2006) The anomalous warming of summer 2003 in the surface layer of the Central Ligurian Sea (Western Mediterranean). Annales Geophysicae 24, 443–452.

Steneck RS, Carlton JT (2001) Human alterations of marine communities: students beware! In: Bertness MD, Gaines SD, Hay ME (eds.). Marine community ecology Sunderland: Sinauer. pp. 159-181.

Taylor BJE (2006) Methods and Procedures for the Verification and Validation of Artificial Neural Networks. Springer, New York.

Tibshirani R (1996) Regression shrinkage and selection via the lasso. J. Royal. Statist. Soc B. 58 (1): 267–288.

Torrents O, Tambutté E, Caminiti N, Garrabou J (2008) Upper thermal thresholds of shallow vs.deep populations of the precious Mediterranean red coral Corallium rubrum (L.): Assessing the potential effects of warming in the NW Mediterranean. Journal of Experimental Marine Biology and Ecology 357: 7-19.

True MA (1970) Étude quantitative de quatre peuplements sciaphiles sur substrat rocheux dans la région marseillaise. Bulletin de l'Institut Oceanographique, Monaco 1410: 1-48.

Vapnik V (1998) Statistical Learning Theory. John Wiley, New York.

Vargas-Yáñez M, Moya F, García-Martínez MC, Tel E, Zunino P et al. (2010) Climate change in the Western Mediterranean Sea 1900–2008. Journal of Marine Systems 82: 171-176.

Vezzulli L, Previati M, Pruzzo C, Marchese A, Bourne DG et al. (2010) Vibrio infections triggering mass mortality events in a warming Mediterranean Sea. Environmental Microbiology 12: 2007-2019.

Walther GR, Post E, Convey P, Menzel A, Parmesan C et al. (2002) Ecological responses to recent climate change. Nature 416: 389-395.

Weinberg S (1975) Écologie des octocoralliares communs du substrat durs dans la région de Banyuls-sur-mer. Bijdragen tot de dierkunde 45: 50-70.

Weinberg S (1980) Autoecology of shallow-water octocorallia from Mediterranean rocky substrata .II. Marseille, Cote d'Azur and Corsica. Bijdragen tot de dierkunde 50: 73-86.

Wellington GM, Strong AE, Merlen G (2001) Sea surface temperature variation in the Galapagos Archipelago: a comparison between AVHRR nighttime satellite data and in situ instrumentation (1982–1998). Bulletin of Marine Science 69:27–42.

Williams G (1966) Adaptation and Natural Selection. Princeton University Press, Princeton.

Wilson RS and Franklin CE (2002) Testing the beneficial acclimation hypothesis. Trends in Ecology and Evolution 17: 66-70.

Worm B, Barbier E, Beaumont N, Duffy JE, Folke C et al. (2006) Impacts of biodiversity loss on ocean ecosystem services. Science 314, 787-790.

Yoshioka PM (1994) Size-specific life history pattern of a shallow water gorgonian. Journal of Experimental Marine Biology and Ecology 184: 111-122.

Younes W, Bensoussan N, Romano JC, Arlhac D, Lafont M (2003) Seasonal and interannual variations (1996-2000) of the coastal waters east of the Rhône river mouth as indicated by the SORCOM series. Oceanologica Acta 26, 311-321.

Yuu Y, Einoshin S, Hideto Y, Katsuhiko T (2003) Decision-tree induction from time-series data based on a standard-example split test. In: Proceedings of the Twentieth International Conference on Machine Learning. Washington DC.

Zabala M, Ballesteros E (1989) Surface-dependent strategies and energy flux in benthic marine communities or, why corals do not exist in the Mediterranean. Scientia Marina 53: 3-17.

Zhang H (1998) Classification trees for multiple binary responses. Journal of the American Statistical Association 93 (441): 180-193.

Zhu J, Rosset S, Zou H, Hastie T (2006) Multi-class AdaBoost. Technical report, Standford University.

Annexes

Annexe 1

Temperature anomalies and mortality events in marine communities: insights on factors behind differential mortality impacts in the NW Mediterranean

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Keywords: seawater temperature, positive thermal anomalies, mass mortality events, coastal rocky benthic community, NW Mediterranean, climate change.

Abstract:

Two large-scale mass mortality events (MMEs) of unprecedented extent and severity affecting rocky benthic communities occurred during the summers of 1999 and 2003 along the coasts of the NW Mediterranean Sea. These mortality outbreaks were associated with positive thermal anomalies. In this study, we performed an analysis of inter-regional and inter-annual differences in temperature (T) conditions associated with MMEs of the red gorgonian *Paramuricea clavata* by analyzing high resolution T time series (hourly records for 3 to 8 years) from four regions of the NW Mediterranean with differing hydrological conditions and biological impacts. High resolution records allowed a detailed analysis using classical and new descriptors to characterize T anomalies. We were able to determine that the MMEs were triggered by two main types of positive thermal anomalies, with the first type being characterized by short periods (2 to 5 days) with high Mean T reaching more than 27^{o}C in some regions and being associated with high intra-day and intra-period variability, while the second type of anomaly presented long duration (near one month) at warm T (24^{o}C) with low intra-period variability. Inter-regional patterns arose; some regions displayed both types of anomalies, while others exhibited only one type. The results showed that T conditions should be considered as the main factor that explains the observed inter-regional and inter-annual differences in mortality impacts. In explaining these differences, the late timing of T anomalies, in addition to their magnitude was found to be determinant. Finally, by combining thermotolerance experimental data with the maximal T stress

conditions observed in the four regions, we were able to determine the differential risk of mass mortality across regions. We conclude that expanding high resolution T series is important for the development of sound management and conservation plans to protect Mediterranean marine biodiversity in the face of climate change.

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Temperature Anomalies and Mortality Events in Marine Communities: Insights on Factors behind Differential Mortality Impacts in the NW Mediterranean

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Introduction

Coastal marine ecosystems harbor high biological diversity and are among the most productive systems in the world [1,2]. These ecosystems are subjected to high levels of anthropogenic pressure, which could have serious implications for the well-being of societies dependent on these ecosystems for goods and services [3].

Overexploitation has been recognized as the major threat to marine ecosystems causing the decline of a number of target species and changes in the structure of food webs [4,5]. However, pollution, invasive species, alteration and loss of habitats and, more recently, global climate change have also been reported to have significant effects in marine ecosystems [6,7,8,9,10].

The analysis of climate change impacts presents a unique challenge for conservation biology because they affect large spatial scales and because they are not easily alleviated by local management actions [11]. Likewise, these impacts affect most levels of biological organization: from population and life-history changes to shifts in the species composition and in the structure and function of ecosystems [10,12]. Therefore, research efforts focused on providing meaningful data for the development of management plans are urgently needed to enhance the resilience of ecosystems facing current environmental changes [8,13].

In the NW Mediterranean (NWM) Sea, recent studies have demonstrated a clear warming trend during the last century and the enhancement of stratification conditions during summer periods in the last 30 years [14,15,16,17]. In this region, warming has been found to be associated with shifts in species distributions [18,19] and mortality events observed during the last 30 years [20,21]. In particular, two recent large-scale (>1000 km of coastline) mass mortality events (MME) of approximately 30 macro-benthic species including sponges, cnidarians, bivalves,

ascidians and bryozoans, occurred during the summers of 1999 and 2003 along the coasts of Spain, France and Italy. In 2006 and 2008, mortality events of a minor extent and severity were also documented in the NWM region [22,23,24]. All these events were associated with positive thermal anomalies [20,21,24,25].

An analysis of the biological impacts of mentioned MMEs has revealed differential responses among species and their populations at all spatial scales considered [21]. At the local level, colonies can show contrasting responses, ranging from severe to a complete absence of injuries. Within regions, populations can display low to high mortality, and there is a clear decrease of impact with depth [26]. As an example, different red coral (*Corallium rubrum*) populations from the same region presented from 5% to 80% of affected colonies [27]. This magnitude of differences has also been observed at the inter-regional level [21]. Finally, the same regions affected by MMEs in different years exhibited differential impacts, both in magnitude and the depth range affected [20,21,25,26].

In this study, we present an analysis of inter-regional and annual differences in T conditions associated with MMEs by analyzing high resolution T time series from four regions of the NW Mediterranean Sea with differing hydrological conditions [22] and biological responses [21]. The characterization of the thermal conditions of different regions and years that displayed mass mortality events and the analysis of corresponding impacts in the red gorgonian *Paramuricea clavata* populations, allowed for the first time the study of the relation of regional temperature conditions with observed impacts at the population level. High resolution T records allowed a detailed analysis using classical and new descriptors. Moreover, the results allowed the discussion of the potential role of temperature conditions and biological factors (e.g. acclimatization, local adaptation) that may underlie the differential impacts of the MMEs.

Materials and Methods

2.1 Study area

The study was conducted in four locations of the NW Mediterranean basin (Fig. 1a), which were the following, from west to east: Parc Natural del Montgrí, Illes Medes i Baix Ter (L'Estartit, Spain); Riou (Marseille, France); Parc National de Port-Cros (France); and Reserve Naturelle de Scandola (Corsica, France) (Fig. 1b). These regions shared the common feature of NW Mediterranean waters of being characterized by a marked seasonality. From late autumn to winter (December–March), the seawater T slowly declines, reaching a minimum in March of approximately 13°C before increasing slightly until the formation of the thermocline [28]. Although they present similarity in their annual T cycles, during summer, the four regions present very distinct hydrographic conditions [22]. Riou exhibits the coldest conditions from depths of 5 to 40 m, while Scandola is the warmest site in its subsurface waters, and Medes and Port-Cros show intermediate conditions. From depths of 15 to 35 m, the warmest T occurs at Medes and Scandola, with Port-Cros being in an intermediate position [22]. With respect to the variability of the

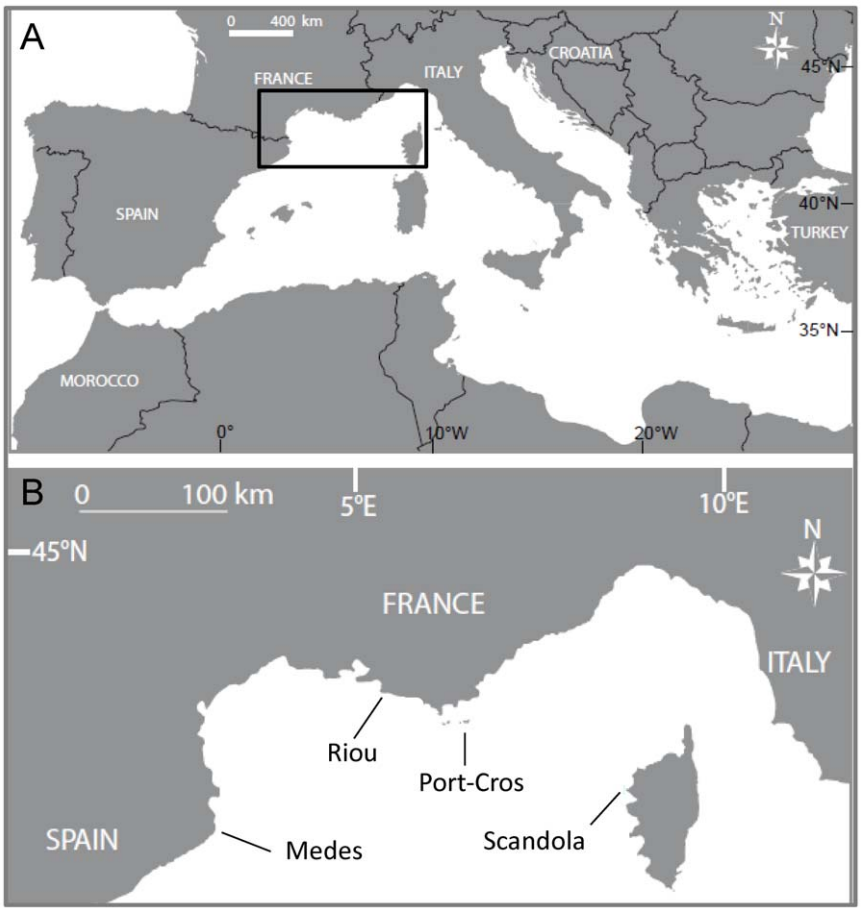

Figure 1. The study area. Northwestern Mediterranean Sea showing the limits of the study area (a) and detail of the NW Mediterranean with the four study regions (b).
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summer thermal regime, inter-regional differences are also observed. Riou is the most variable site from depths of 5 to 40 m because of the occurrence of upwelling, while Medes exhibits the highest variability at 40 m because of the recurrent downwelling. Finally, Port-Cros and Scandola display the maximum variability at 25–30 m because of oscillations of the thermocline that settles around these depths [22].

2.2 Temperature measurement strategy

In each region, T records were registered by *in situ* Stowaway Tidbits autonomous sensors set up in sites exposed to dominant winds and currents.

The recorded period was from 1999 to 2006. Records began in June 1999 at Riou and Port-Cros, in July 2002 at Medes and in April 2004 at Scandola. Since 2004, measurements have been standardized at all regions to collect hourly records. Previously, T measurements had been recorded every 2 hours, and these time series were interpolated using an exact interpolation technique to obtain a set of synchronous hourly data. T data were not available for all years within each region; the available periods of T measurements at each depth are shown in Table 1. These temperature series were previously used to characterize temperature regimes in the four studied regions ([22]).

2.3 Biological data collection

Biological surveys were conducted in the four study regions to investigate relationships between temperature conditions and population responses. For this purpose, we chose populations of the red gorgonian *Paramuricea clavata* as model species because the largest dataset was available for this species, and it was one of the most affected during the MMEs [20,21,25]. Furthermore *P. clavata* is considered one of the key species of Mediterranean coralligenous assemblages ([29],[30]). Since other macrobenthic species affected by the MMEs showed similar inter-regional and inter-annual pattern of mortality to *P. clavata*, the patterns obtained for this species may be considered representative of the MMEs impacts for other species as well ([20],[21], authors unpublished data). For all these reasons we contend that the use of *P. clavata* provide an excellent model for the analysis of the relationships between temperature conditions during temperature anomalies and biological impacts.

During the surveys, the percentage of recent tissue necrosis (i.e., denuded axis or recent epibiosis) was quantified in at least 100 colonies present at each site and depth surveyed.

We considered a colony to be affected by mortality when it showed recent tissue necrosis over 10% of its surface. Finally, for each survey, the percentage of affected colonies was calculated as an indicator of the mortality impact (see [21] for further information). Surveys were conducted on an annual basis at 2–7 sites within each study area. In cases where a MME was observed, the number of sites was increased when possible to better quantify the mass mortality impacts. More than 20.000 colonies were analyzed within the four study regions. We calculated the percentage of affected colonies within each region at every year that presented mass mortality events and inside each region we averaged the values of the years that not displayed mortality outbreaks. Percentages were calculated separately for 10 and 25 m depth ranges. In Port-Cros and Scandola, the surveys only concerned the 25 m depth because at 10 m, *P. clavata* populations are absent or show low abundance [26,31].

Kruskal-Wallis analysis was used to test for differences of mortality rates between all regions, years and depths. Multiple comparisons were studied through Mann-Whitney tests to determine specific differences between pairs of data. Nonparametric tests were selected because of the absence of normality and homoscedasticity in the dependent variable. The Kruskal-Wallis and Mann-Whitney tests were computed using PAST software (version 1.82b, [32]).

2.4 Characterization of temperature anomalies related to mass mortality events (MME)

To characterize the temperature conditions of years associated with mortality events in each region, we combined classical and new descriptors to retain information on the magnitude, variability and duration of T anomalies, as well as the timing of the anomalies during the summer period (see below).

In the analysis, the period between 1st July and 30 September was arbitrarily considered as the summer period. Likewise, the depths considered were 10 and 25 m (12 and 24 m at Riou and Port-Cros in the 1999–2003 period, hereafter referred to as 10 and 25 m, respectively), which correspond to the suprathermoclinal and intermediate thermoclinal levels, respectively [22]. We selected this period and these depths because the MMEs displayed the most severe impacts under these conditions [20,21,23,25].

Finally, we distinguished two types of years: those associated with mortality events in at least some of the studied regions (hereafter YMMEs), which included 1999, 2003 and 2006; and those presenting no mortality event signals (hereafter YNMMEs), which included 2000, 2001, 2002, 2004 and 2005. For all of the analyzed statistics, for each location and depth, each YMME was analyzed separately, while YNMMEs were studied together, averaging statistical values. Representation and analysis of the data were performed using SigmaPlot (version 10.0) and PAST (version 1.82b, [32]) software, respectively.

2.4.1 Mean T, maximum T and coefficient of variation of the summer period. The Mean T, maximum T (Max T) and coefficient of variation (CV) were calculated to search for differences between YMMEs and YNMMEs and within YMMEs. The CV (summer standard deviation×100/summer Mean T) was chosen as the measure of variability because it is the

Table 1. Availability of temperature data.

Geographic region	10 m	25 m
Parc Natural del Montgrí, Illes Medes i Baix Ter (L'Estartit, Spain)	2003–2006*	2003–2006
Riou (Marseilles, France)	1999–2006	1999–2006
Parc National de Port-Cros (France)	1999, 2001–2006	1999–2000, 2002–2006
Reserve Naturelle de Scandola (Corsica, France)	2004–2006	2004–2006

Available temperature data for the four study regions in the northwestern Mediterranean Sea at 10 and 25 m depths (*: temperature at 15 m depths was analyzed instead of temperature at 10 m). The selected depths include the depth range in which the most severe impacts of MMEs were observed.
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percentage of the degree of variability and can be interpreted independently from the mean.

2.4.2 Mean and CV of time intervals with the highest Mean T. Consecutive episodes of 2, 5, 10, 15, 30 and 40 days (taking 24 consecutive hours as a day, 48 consecutive hours for two days, and so on) with the highest Mean T were retained. For each year, region and depth, there was a unique corresponding consecutive period of a specific length with the highest Mean T. Thus, the value of this Mean T and the corresponding value of the CV of each length period were retained with the aim of capturing the T magnitude and associated variability of the hottest periods of short, intermediate and long duration.

2.4.3 Timing of periods with the highest Mean T. The timing of the above-mentioned 15- and 40-day periods was analyzed. Timing refers to the point in time in the summer when these periods occurred. For the 15-day episodes, summer was divided into 6 two-week summer periods: 1st–15 July, 16–31 July, 1st–15 August, 16–31 August, 1st–15 September and 16–30 September. Then, the 15-day episodes with highest Mean T were associated with the two-week period in which most of the episode occurred. For the 40-day episodes, the summer period was divided into months: July, August and September. Again, the 40-day episodes with highest Mean T were associated with the month in which most of the episode occurred. By analyzing the timing of the temperature anomalies, we intended to explore the response of affected species to similar temperature stresses occurring at different times during the summer.

2.5 Ordination of YMMEs and YNMMEs through T statistics

The analyzed statistics (except those on temperature anomalies timing) were ordered through Principal Component Analysis (PCA) with the aim of synthesizing the information provided by the different T indicators. Two analyses were performed, one for the 10 m depth and another for the 25 m depth. In addition to the analyzed statistics described above, three further variables were considered to perform the PCA. We included, in one side, the total duration (as the proportion of summer time) inside the [24–25] °C T class and the longest consecutive duration inside this class. These statistics were considered since it was documented that during 1999 summer, long duration near 24°C occurred at Riou and at Port-Cros ([22,31]). In the other side we included the average CV of the 5 hottest summer days. It was calculated averaging the CV value corresponding to the 5 summer days with the highest Mean T. This indicator was included with the aim of considering variability of the thermal regime at shorter time scales (e.g. inside days). Overall, a total of 15 variables were available to perform the analyses, but because of the very high correlation between some of them (Pearson correlation coefficient >0.8), redundant variables were removed to perform PCAs. Finally, a total of six and eight variables were retained to perform the 10 and 25 m depth PCA, respectively. For the 10 m depth PCA the retained variables were the Mean T of the 5 and the 40 consecutive days with highest mean T (Mean_T_5 and Mean_T_40 respectively), the CV of the 5 and the 40 consecutive days with highest mean T (CV_5 and CV_40 respectively), the average CV of the 5 hottest summer days (Mean_CV_5) and the total duration inside the [24–25] °C T class (Dur_24). For the 25 m depth PCA all the variables retained for the 10 m depth PCA, the CV of the 15 consecutive days with highest mean T (CV_15) and the longest consecutive duration inside de [24–25] °C T class (Max_cons_dur_24) were analyzed.

As for the previously calculated statistics, YNMMEs were considered together, while YMMEs were studied separately.

2.6 Confronting thermotolerance experiment results and field T conditions

The available information from the experimental results on the thermotolerance of NWM rocky benthic species (Table 2) was contrasted with the most severe field T conditions observed in the four studied regions. An inverse second-order regression ($f = y0+(a/x)+(b/x^2)$) was fitted to the Mean T of increasing time intervals (from 2 to 40 days) with the highest Mean T with the aim of obtaining a domain of possible conditions in each region given the available datasets and comparing them with the upper thermotolerance limits from experimental data. Given that points located in domains below the regression curves indicate species at risk under actual conditions, we suggest that differences with respect to the distribution of experimental data above and below the regression curve could provide clues related to the differential risk of mortality among regions.

Results

3.1 Biological data

Clear differences were found in the mortality rates associated with different years, regions and depths, and these differences were significant (Kruskal-Wallis p-value<0.05). The highest mortality rates were found at Riou in 1999 at 10 and 25 m depths, at Riou in 2003 at the 10 m depth and at Port-Cros in 1999 at the 25 m depth, which all presented between 23 and 46% affected colonies (Fig. 2). Multi-comparison analyses did not indicate significant differences among these observations (p-value>0.05). These comparisons also showed that the cases with high mortality rates presented significant differences compared with all remaining cases, which experienced low to zero mortality rates (p-value<0.05). However, there was an exception for Riou in 2006 at the 10 m depth, which did not present differences with Port-Cros in 1999 at the 25 m depth. At this time and depth, Riou presented nearly 10% affected colonies, which was a value that was significantly higher than those of observations associated with low mortality rates (p-value<0.05, Fig. 2).

3.2 Characterization of the summer thermal regime of YMMEs

3.2.1 Mean T, maximum T and coefficient of variation of the summer period. At 10 m depths, the highest summer Mean T was observed in 1999 at Riou and Port-Cros and in 2003 at Medes (Table 3). For this year at these regions, the highest Mean T values were more than 1°C warmer than those in all remaining years. In particular, this value for Riou in 1999 was almost 2.5°C warmer than for YNMMEs. Finally, Scandola did not present important differences between 2006 (the only YMME

Table 2. Availability of experimental data.

Species	Tested T (°C)	References
Cladocora caespitosa	24, 26,	[56]
Corallium rubrum	24, 25, 27	[38,39]
Eunicella singularis	24	[57]
Oculina patagonica	24, 26,	[56]
Paramuricea clavata	23, 24, 25, 25, 27	[16,37,39], Crisci et al. unpublished data

Data from experimental work on T effects on mortality (necrosis) of the NWM rocky benthic species used to produce Figure 7.
doi:10.1371/journal.pone.0023814.t002

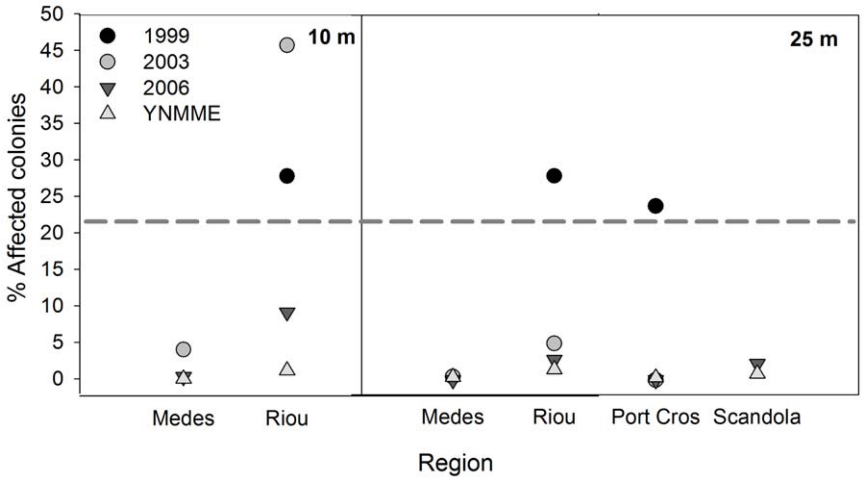

Figure 2. Mortality of *Paramuricea clavata*. Percentage of affected colonies with ≥10% of the colony surface showing recent necrosis (denuded axis or recent epibiosis) for *Paramuricea clavata* populations in the four study regions of the northwestern Mediterranean sea at 10 and 25 m depths during 1999, 2003, 2006 and YNMMEs. The cases with the highest rates of necrosis are those above the grey dashed line. Within this group of data there were no statistical differences in mortality rates (p-value>0.05), while % of affected colonies showed statistical differences with the remaining years, regions and depths (p-value<0.5). There was an exception for Port-Cros in 1999 at the 25 m depth, which did not present differences with Riou in 2006 at the 10 m depth.
doi:10.1371/journal.pone.0023814.g002

with T data available) and YNMMEs. The Max T was reached in 2003 at Medes, Riou and Port-Cros and in 2006 at Scandola. In this last region and year, the highest Max T of all regions and years was observed, reaching almost 28°C (Table 3). Additionally, either 2003 or 2006 was the year that presented the highest CV in all regions. The high values of the CV in Riou are remarkable in both YMMEs and YNMMEs in comparison with the other regions (Table 3).

At the 25 m depth, as at the 10 m depth, the highest Mean T in Riou and Port-Cros were found in 1999 (1 to 2°C higher than other years). No remarkable differences were found among years for the other regions. However, in relation with the Max T, clear differences were found between Medes in 2003 and 2006 compared with YNMMEs (up to 2°C warmer) and Port-Cros in 1999 compared with all other years (1.3°C warmer). In contrast, in Scandola in 2006, the Max T was 1°C colder than in YNMMEs, indicating enhanced stratification, with warm T limited to shallow depths at this location. In Medes, 2003 and 2006 were more variable than YNMMEs, while 1999 and 2003 presented higher CVs than 2006 and YNMMEs in Port-Cros (Table 3).

3.2.2 Mean T and CV of consecutive time intervals with the highest Mean T. In general, at the 10 m depth, YMMEs presented a higher Mean T than YNMMEs, regardless of the time interval considered (Figs. 3a–d). However, inter-annual and inter-regional differences were observed. Medes in 2003 and 2006 (Fig. 3a) and Riou and Port-Cros in 1999 (Figs. 3b and 3c) presented remarkable constancy throughout all time periods considered compared with YNMMEs, reaching a Mean T near 24°C in the longest periods and a T near 25°C in the shorter periods considered. On the other hand, clear differences with YNMMEs were also found in the Mean T for short periods at Medes and Riou in 2003 and 2006 and at Scandola in 2006, where the Mean T values recorded were approximately 2–3°C higher than the values found in YNMMEs (Figs. 3a, 3b and 3d).

Changes in the CV for the different time intervals were also observed during YMMEs at 10 m depth (Figs. 3e–h). In Riou and Port-Cros in 1999, the constancy in the Mean T over the different time intervals was reflected in a drastic decrease in the CVs compared with other years (10 and 5.5%, respectively, for the longest episodes) (Figs. 3f and 3g). For the other investigated years, Riou and Port-Cros showed CV values similar to those for YNMMEs, except in Port-Cros in 2003, where the CV for short periods was greater than for YNMMEs. In Medes and Scandola, YMMEs were characterized by an increase of the CV, especially when long periods of time were considered (Figs. 3e and 3h).

At the 25 m depth, the Mean T of YMMEs versus YNMMEs did not differ as strongly, and it was warmer for all time periods examined only in Medes in 2006 and Riou and Port-Cros in 1999 (Figs. 4a–c). Medes in 2003 presented a higher T than YNMMEs, but only for short-length (≤5 days) episodes (Fig. 4a). All other years and regions did not present remarkable differences with YNMMEs (Figs. 4a–d). The most important differences compared with YNMMEs (2 to 4°C) were observed in Port-Cros in 1999, which presented a notable constancy in exhibiting a relatively high T throughout all time periods considered, reaching Mean T from 23.5°C to 24.5°C for the longest and the shortest periods, respectively (Fig. 4c). A similar pattern, though mainly concerning intermediate and long episodes, was observed in Riou in 1999 (Fig. 4b).

Because of the relative constancy in the 1999 Riou and Port-Cros thermal conditions, the CV was lower than that in all remaining years during intermediate and long episodes in Riou (Fig. 4f) and for long episodes in Port-Cros (Fig. 4g). The remaining years and regions did not differ significantly from YNMMEs (Figs. 4e–h).

3.2.3 Timing of consecutive time intervals with the highest Mean T. At the 10 m depth, the 15-day episodes with the highest Mean T were relatively well segregated according to years (Fig. 5a). During 2006, these episodes occurred between the beginning and the middle of summer (16–31 July in Riou and Port-Cros and 1st to 15 August in Medes and Scandola). In 2003, they occurred mainly in the middle of the summer period (1st to 15 August in Riou and 16 to 31 August in Medes and Port-Cros), and in 1999, they occurred late in the summer period, between 1st and 15 September (Fig. 5a). For YNMMEs, the warmest 15-day consecutive episodes occurred in August, though there was a great deal of variability depending on region (Fig. 5a).

Table 3. Classical T descriptors.

Region	Year	Mean summer T (°C)	Max. summer T (°C)	Summer CV (%)	Mean summer T (°C)	Max. summer T (°C)	Summer CV (%)
		10 m depth			25 m depth		
Medes	2003	22.3	25.5	8.4	18.3	24.5	15.2
	2006	21.6	25.0	10.1	19.6	24.9	14.3
	YNMME	21.2±0.7	23.4±1.1	4.6±1.4	19.5±0.3	22.9±0.9	9.9±3.3
Riou	1999	21.4	25.0	15.1	19.4	24.1	17.4
	2003	20.3	27.6	17.7	17.5	24.2	13.4
	2006	20.2	26.8	16.2	18.3	23.6	15.0
	YNMME	19.0±0.3	24.7±0.9	17.5±1.2	17.5±0.3	23.8±1.3	15.9±2.4
Port-Cros	1999	22.8	25.5	8.3	20.3	25.1	15.0
	2003	21.7	27.2	12.8	18.1	23.0	13.6
	2006	22.0	26.5	8.8	18.9	23.1	9.3
	YNMME	21.4±0.9	24.9±0.8	8.9±1.6	18.4±0.6	23.8±0.4	12.1±1.1
Scandola	2006	23.0	27.9	7.0	19.7	23.8	9.3
	YNMME	23.3±0.4	25.7±0.5	4.8±1.3	19.9±0.7	24.8±0.5	12.0±0.3

Mean T, maximum T (Max T) and coefficient of variation (CV) for the summer period of the four study regions for 1999, 2003, 2006 and YNMMEs (mean ± SD) at 10 and 25 m depths.

doi:10.1371/journal.pone.0023814.t003

When 40-day consecutive episodes were considered, 1999, 2003 and 2006 were again well segregated. These episodes occurred in July during 2006, in August during 2003 and in September during 1999. During YNMMEs, they were mainly distributed in the middle of the summer, although great variability was observed within some regions (Fig. 5b).

At 25 m, both 15- and 40-day episodes occurred first in Medes 2006 (between 1st and 15 August and in July, respectively), while for all other YMMEs, episodes of both lengths occurred during September (Figs. 5c and 5d). YNMMEs presented intermediate and long hottest episodes, mostly at the end of the summer and always earlier than for YMMEs, although variability within some regions was observed (Figs. 5c and 5d).

3.3 Ordination of YMMEs and YNMMEs through T statistics

Considering the PCA for the 10 m depth, the first two axes accounted for 77% of the variance of the data. The first axis, which retained 42% of the variance, was useful for discriminating two main types of T anomalies. Riou and Port-Cros in 2003 and Scandola and Riou in 2006 were positively associated with this axis (Fig. 6a). Projection of the original T variables illustrates the summer thermal characteristics of these regions and years, which were associated with high T during short periods of time (five days) and with large hourly variability during the hottest summer days. High variability within short, intermediate and long hottest episodes was also a feature of these years. Negatively associations with the first axis were found for Riou and Port-Cros in 1999 and, with lower associated scores, Scandola in YNMMEs and Medes in 2003 and 2006. With the exception of Scandola in YNMMEs, these years represent a second type of thermal anomaly. This type of anomaly was characterized by long total and consecutive durations during warm T as well as by low variability at all time scales considered (Fig. 6a). The position of Scandola YNMMEs could be explained because the subsurface waters of this region are the hottest among the four regions (Table 3).

The second axis accounted for the characteristics of YNMMEs (with the exception of Scandola YMMEs), which were mainly low Mean T of short and large consecutive episodes with the highest Mean T (Fig. 6a).

At the 25 m depth, the first two axes accounted for 71% of the variance of the data (Fig. 6b). The first axis (51% of variability) distinguished the years that presented anomalous thermal regimes from those that did not. These years were 1999 for Port-Cros and Riou and 2006 for Medes and were characterized by a relatively long duration within warm T and relatively high T in short and long intervals with the highest Mean T. The second axis explained low data variability and did not clearly segregate YMMEs from YNMMEs. It was associated with variability related to different length episodes and separated regions inside YMMEs and YNMMEs (Fig. 6b).

3.4 Confronting thermotolerance experiment results and field T conditions

The distribution of the experimental thermotolerance data points around the field T data curves provided clues related to the degree of vulnerability of the different species under recent T conditions in the 4 regions (Fig. 7). The experimental results indicated that short to moderate exposure (1 to 14 days) to 25°C and short exposure to 26 and 27°C (1 to 3 days) could lead to mortality (with the exception of the symbiotic species *C. caespitosa* and *O. patagonica*, which seem to be more resistant to these T conditions). These temperature conditions were attenuated in Medes and Port-Cros regarding exposure to 25°C, and there was very low or nonexistent exposure to 26 and 27°C at these sites. Conversely, these conditions were more frequent in Riou and Scandola for YMMEs, reaching longer durations at 25 and 26°C and, in the case of Scandola, also at 27°C (Fig. 7). Therefore, Medes and Port-Cros appear to represent the less risky of the investigated regions, while in contrast, Riou and Scandola appear to be the most risky regions in terms of higher chances of experiencing mortality.

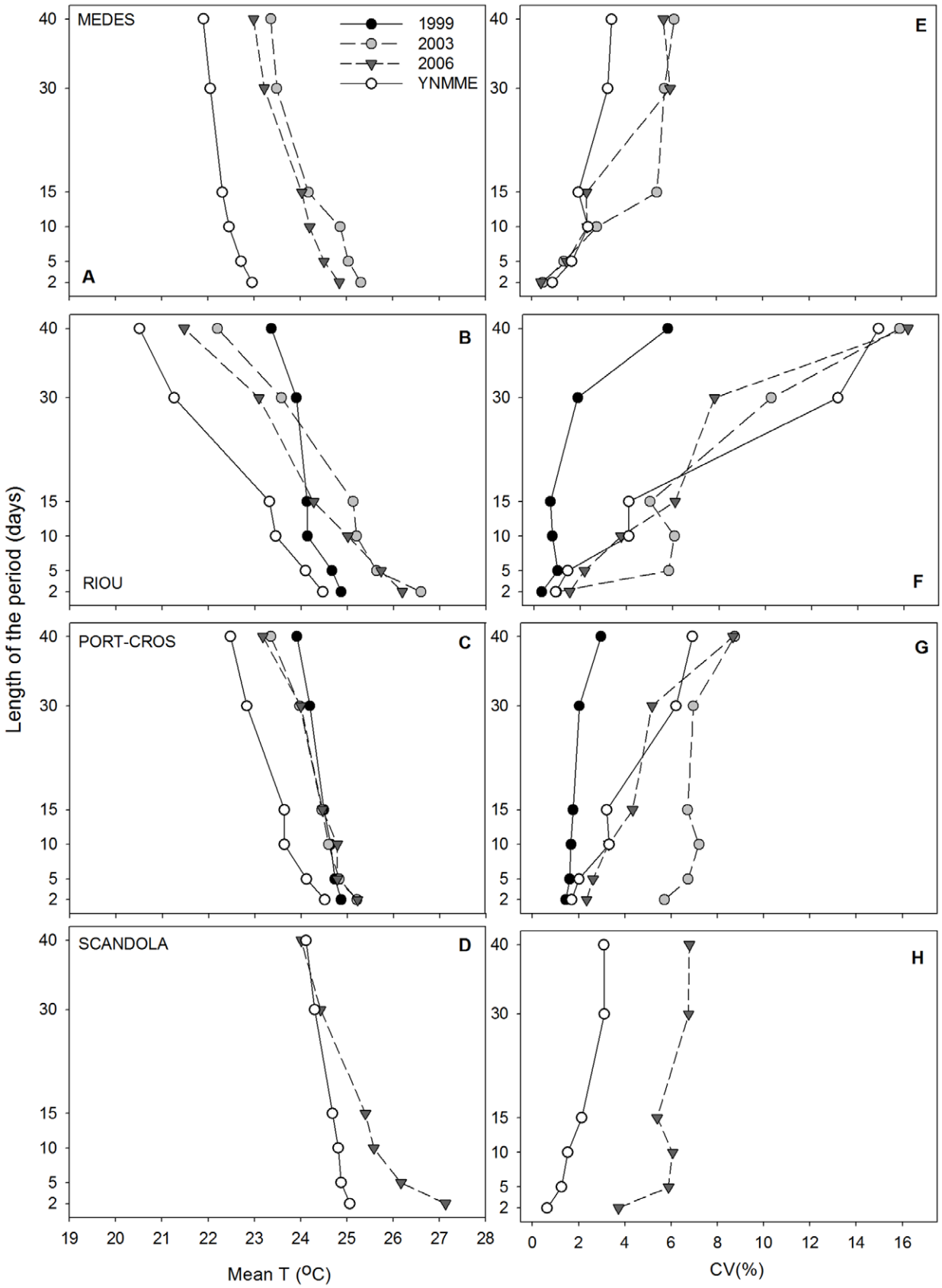

Figure 3. Mean T and CV of consecutive episodes with the highest mean T (10 m depth). Mean temperature (°C) and coefficient of variation (%) of consecutive episodes of 2, 5, 10, 15, 30 and 40 days with the highest mean temperature. Data are presented for the four study locations at 10 m depths.
doi:10.1371/journal.pone.0023814.g003

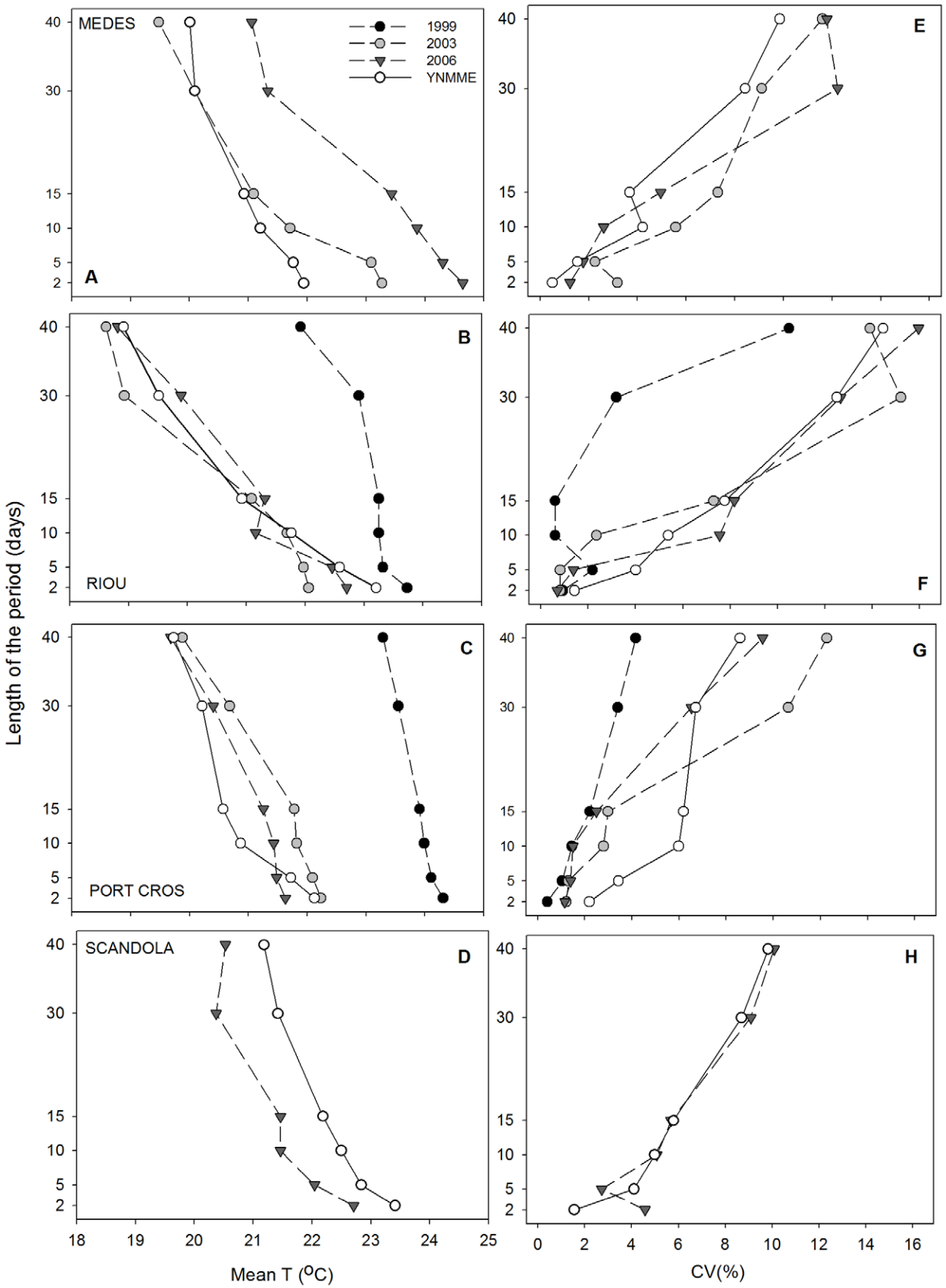

Figure 4. Mean T and CV of consecutive episodes with the highest mean T (25 m depth). Mean temperature (°C) and coefficient of variation (%) of consecutive episodes of 2, 5, 10, 15, 30 and 40 days with the highest mean temperature. Data are presented for the four study locations at 25 m depth.
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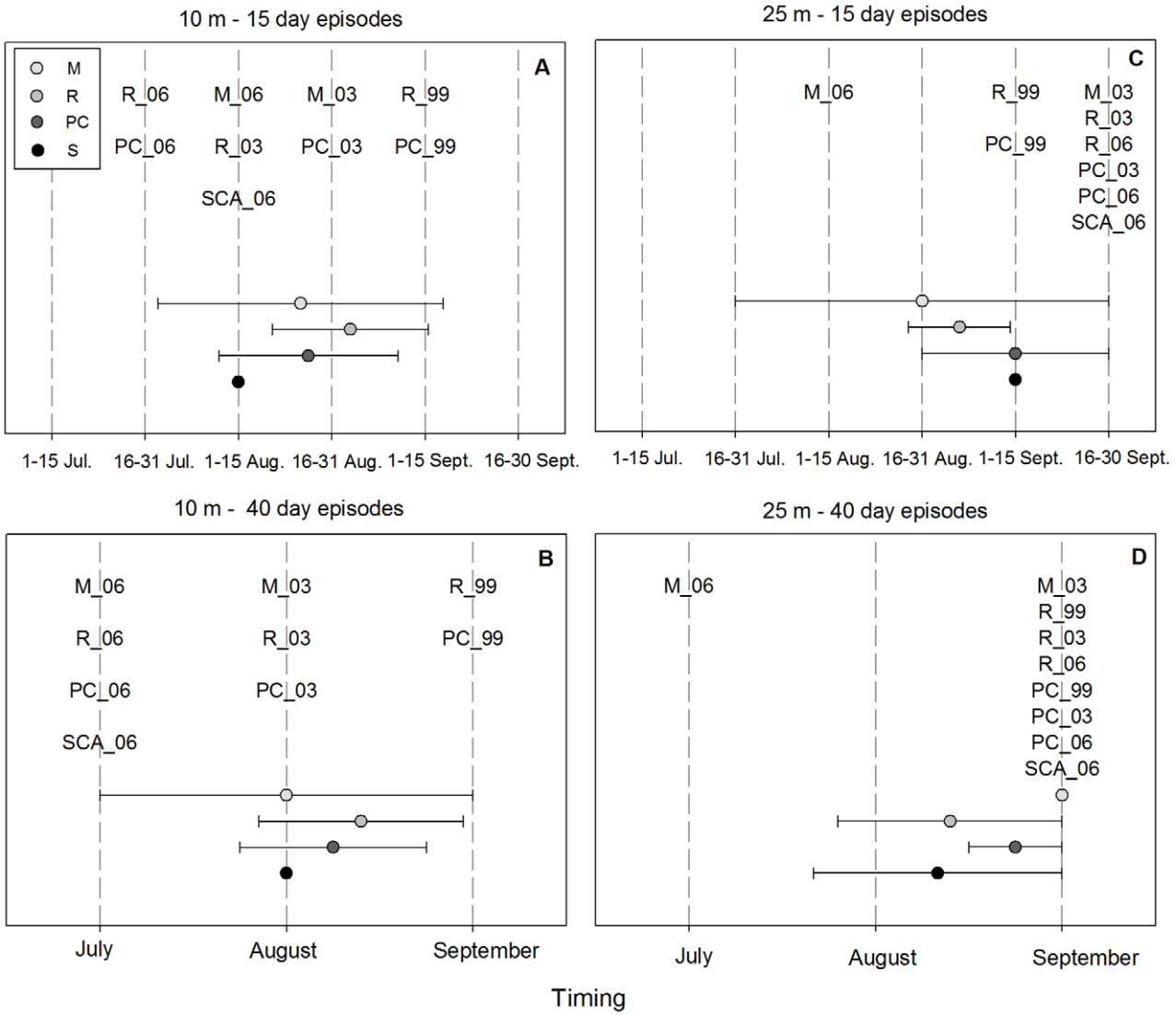

Figure 5. Timing of the 15 and 40 consecutive days with the highest mean T. Timing of consecutive episodes of 15 (a, c) and 40 days (b, d) with the highest mean T for 10 and 25 m depths. The average ± SD is presented for YNMMEs. Cases were ordered in the Y axis to facilitate data reading, but their position in this axis does not provide any information. M: Medes, R: Riou: PC: Port-Cros and S: Scandola.
doi:10.1371/journal.pone.0023814.g005

Discussion

In this study, we addressed for the first time the relationship of inter-regional and inter-annual differences in temperature conditions with the observed impacts in the macrobenthic populations of the NWM Sea during mass mortality outbreaks.

We were able to recognize two types of thermal anomalies that were likely to trigger an MME. The first type was characterized by short episodes (2–5 days of duration) with high Mean T, which were near 27°C in some regions, and high intra-day and intra-period (2–5 days) variability. The second type of anomaly presented long periods (30–40 days) with warm Mean T of approximately 24°C associated with low intra-period variability.

We found inter-regional and inter-annual differences in the occurrence and characteristics of the recorded anomalies. Riou and Port-Cros showed both types of anomalies: long term in 1999 and short term in 2003 and 2006. Furthermore, Medes displayed only long-term anomalies in both years in which anomalies were analyzed (2003 and 2006). Finally, in Scandola, a short-term anomaly was observed in the only year with anomalous T conditions (2006) for which data were available. It is worth noting that both in 2003 and in 2006, the two types of anomalies occurred simultaneously among regions, indicating that two of the largest heat-waves to ever peak over southern Europe [33,34] resulted in differential anomalous warming conditions in the water column. These differences could be attributed to the distinct summer hydrological conditions found in the four study areas [22].

In Medes, there are recurrent downwellings (>40 m) during summer [22], which carries increased T further down in the water column, resulting in warm conditions over longer periods, but never reaching the elevated temperatures found in other study areas. In Riou and Port-Cros, the hydrological conditions can experience abrupt changes under the influence of Mistral (NWN) winds [35]. The lower frequency and shorter duration of these winds prevent the upwelling of deep, cold waters and the subsequent breakage of the thermocline, thus allowing the T to increase [22]. Scandola exhibits stable summer stratification conditions [22], and thus, the high air temperature and calm

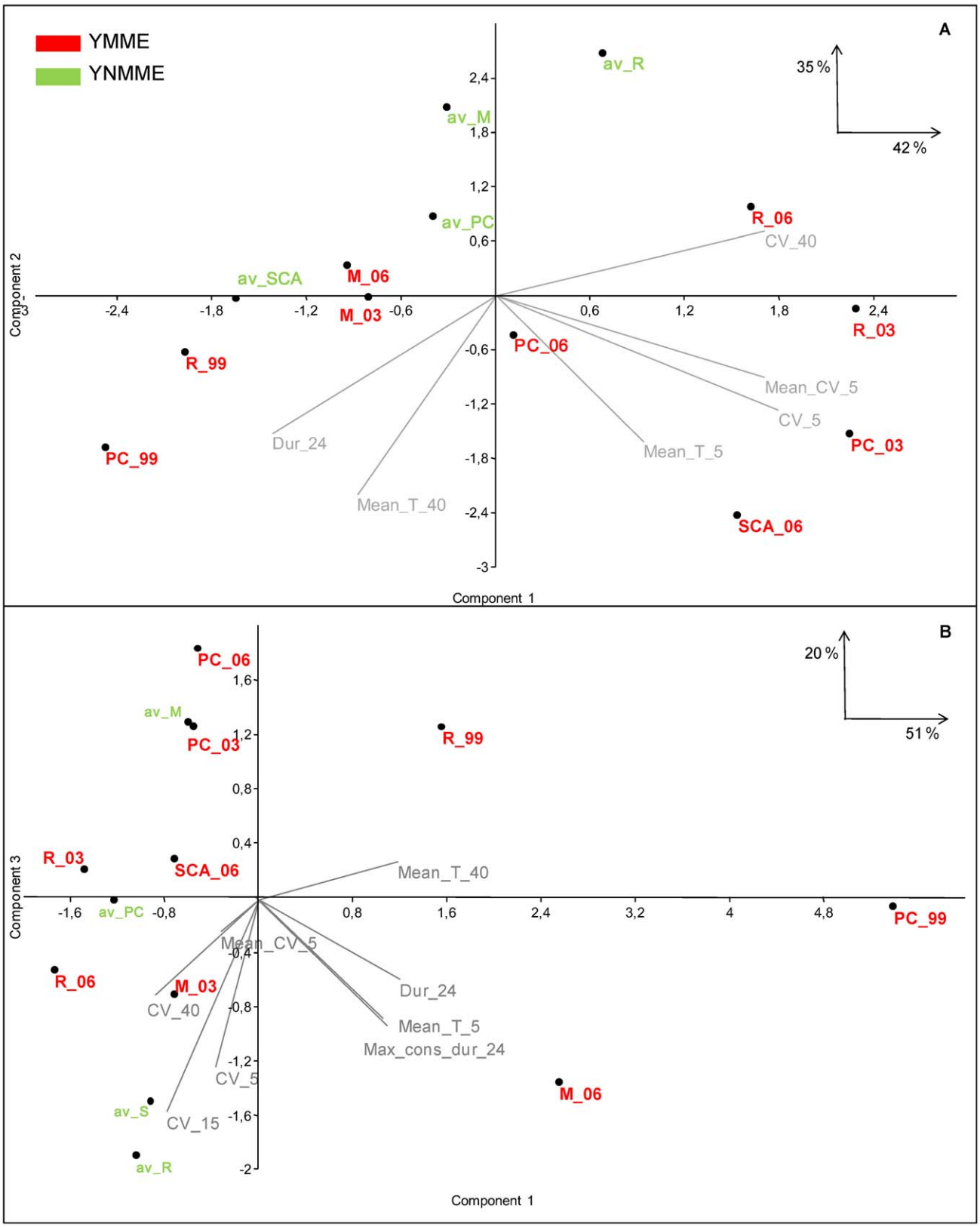

Figure 6. Principal Component Analysis representation. Representation of the first two axes of the Principal Component Analysis using T data from 10 (a) and 25 m depths (b). Two first axes were retained at both depths, accounting for 77 and 71% of data variability. The original T variables used to perform the analyses are represented in grey. Scandola in 2004 at the 10 m depth was not considered among the Scandola YNMMEs in the PCA because this location and year presented the particularity of reaching high temperatures (but not as high as for YMMEs at this location) with no mortality, so it affected the data ordination when considering all locations together. M: Medes, R: Riou: PC: Port-Cros and S: Scandola.
doi:10.1371/journal.pone.0023814.g006

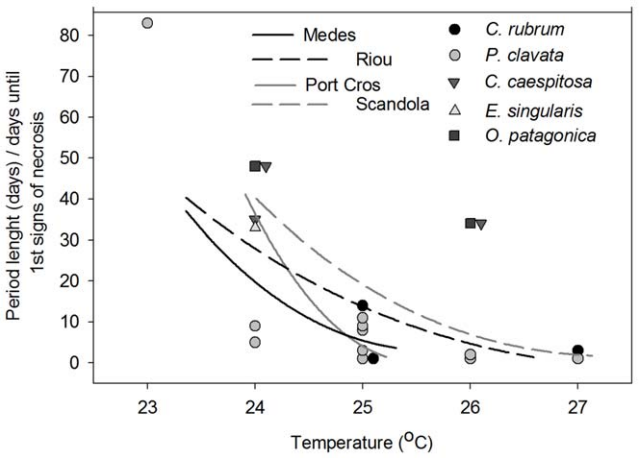

Figure 7. Field T versus experimental thermotolerance data. Inverse second order regression (f = y0+(a/x)+(b/x^2) fitted to field T data (highest mean T of different length episodes) of the four study regions at 10 m depth (complete and dashed lines) and experimental results (day of 1st signs of necrosis) from different rocky benthic species (grey and black symbols) obtained from the bibliography and the authors' unpublished data (see Table 2). For *E. singularis* and *O. patagonica* at 24°C and for *O. patagonica* at 26°C, the number of days until the 1st signs of necrosis actually indicates the number of experimental days since no necrosis was observed until the last day. The aim of the figure is to represent a composite function over which, under experimental conditions, the species seems to tolerate field T regimes and under which species are affected by tissue necrosis.
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weather conditions recorded during the 2006 heat wave resulted in a significant T increase at the suprathermoclinal level. The occurrence of the long- and short-term anomalies recorded can likely be mainly attributed to the timing of calm conditions in the early-middle for 2003 and 2006, and late summer for 1999. The observed differences in the magnitude of both types of T anomalies were able to explain the differences in mortality rates observed in the field. Short periods of high T and long periods of warm T were associated with high mortality rates, being the attenuation of these characteristics (short periods of moderate T or shorter periods of warm T) linked to a significant decrease of mortality rates. Finally, years without thermal anomalies presented negligible mortality rates. Despite these relationships, it is important to precise that within the same region populations experiencing the same thermal regime presented different mortality impacts during MME ([21],[27]), indicating, thus, that other factors than T may be involved in modulating the mortality impacts (see below).

The temperature conditions associated with T anomalies may be related to different biological mechanisms resulting in the death (total or partial) of organisms. In relation to long-term anomalies, the highest mortality rates were observed when long periods of warm Ts occurred at the end of the summer. Mediterranean suspension feeder species exhibit energetic constrains during summer [36], since these organisms have to cope with high respiration demands because of warm T during a period of food scarcity [16]. When these conditions are prolonged, as in the case of the years with anomalies, the organisms can suffer physiological stress that can ultimately lead to partial or total death of some specimens [16]. In previous studies, experimental data demonstrated that long duration exposure to warm T ($\sim$23°C for >40 days), similar to the conditions observed during long-term anomalies, could cause the appearance of the firsts signs of necrosis [16]. In the same experiment, when the colonies were fed, the time of exposure to warm T before observing necrosis almost doubled, clearly indicating that feeding helps to cope with physiological stress [16]. Therefore, the physiological status of organisms is important in modulating their response to thermal stress. This factor may be behind the observed differential mortality observed during long-term anomalies in Riou and Port-Cros in 1999 and Medes (2003 and 2006).

The high T observed during short-term anomalies reached lethal levels for the benthic species of the study regions, as demonstrated through thermotolerance experiments with different Mediterranean gorgonian species ([37,38,39], Crisci et al., unpublished data). Additionally, the high intra-day and intra-period variability that characterized this type of anomaly could be an additional stress factor on the organisms, as found in some tropical coral species [40,41]. Nevertheless, when this type of anomaly occurred at the beginning of summer, such as those that took place in 2006, they resulted in less severe impacts on these populations, probably because the species were less affected by energetic constraints during this period [16].

Finally, for both types of T anomalies, the induction of mortality being provoked by thermo-dependent pathogens cannot be discarded, considering that experimental and field data demonstrated that Ts$\geq$22°C promote pathogen virulence and/or increase host susceptibility [23,37].

Overall, the results of this study indicated that inter-regional differences in mortality rates should be mainly attributed to differences in the T conditions recorded in each region and year with a T anomaly. However, because the populations inhabiting the studied regions were subjected to different magnitudes and timing of T anomalies, we could not determine the potential role of biological factors, such as acclimatization [42], local adaptation [43,44] or even contemporary evolution [45], previously highlighted for other marine species [46,47,48]. Bearing in mind that most of the species affected by the MME inhabit different thermal regimes within the NW Mediterranean basin [22,28] and appear to be characterized by significant genetic differentiation, even at reduced spatial scales of several meters [49,50], it seems likely that selective processes could play a role in determining their response to T anomalies. The available experimental data suggest an inter-depth differential response to the same experimental T ([38,50]). To further explore the role of selective processes in these phenomena, regional-scale thermotolerance experiments should be conducted.

The combination of field T data on the most extreme observed Ts of each studied region with available thermotolerance data on NW Mediterranean anthozoan species allowed us to assess the risk of suffering MMEs in the different study regions (Fig. 7). In general, the T conditions observed in Medes, which exhibits an absence of extreme T (short-term anomaly) and attenuated characteristics of long-term anomalies, do not reach values causing severe damages to these organisms. Therefore, this region could be associated with a lower risk of mortality outbreaks compared with the other regions under present T conditions. In contrast, Riou and Scandola appeared to be the regions with the highest risk, as in both cases, the anomalous T conditions reached values beyond the tolerance of the species addressed in this study. The case of Riou is interesting because it is located in one of the coldest areas of the NW Mediterranean [51]. The fact that both types of anomalies could occur in this area, combined with the shallow distribution of species affected by MMEs [20,21] leads to an unfavorable scenario for Riou populations in the future. Scandola presented the most extreme T of short and intermediate-length periods, which makes it difficult for species affected by MMEs in

shallow depth ranges to survive in this region. In fact, the absence of *P. clavata* populations at the 10 m depth in this area could indirectly suggest that T could be modulating this species' depth distribution, although effects of other environmental factors cannot be discarded (light, water motion, food availability) [52,53]. Finally, Port-Cros occupied an intermediate position in terms of the risk of presenting MMEs, mainly because of the absence of high T episodes. In accordance with these results, the number of species affected and the incidence of mortality during MMEs showed the lowest values in Medes, followed by Port-Cros and, finally, by Riou and Scandola [20,21,25].

In this study, we demonstrated the utility of acquiring and analyzing high resolution T series, which allowed us to determine the main T conditions responsible for the differential mortality impacts observed in the NWM basin and to assess the risk of MMEs in the studied regions. The acquisition of new high resolution T time series in different regions of the Mediterranean (e.g., /T-MEDNet, http://t-mednet.org) will allow expanding the analysis to better characterize and understand current shifts in environmental conditions at larger spatial scales. Additionally, under the present warming scenario for the Mediterranean area [54], these data will be key components in the development of MME risk maps at the scale of the NW Mediterranean. This information is urgently needed to develop sound management and conservation strategies to face the impacts of climate change on the rich marine biodiversity in the Mediterranean region [55].

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Author Contributions

Analyzed the data: CC NB J-CR. Contributed reagents/materials/analysis tools: CC NB. Wrote the paper: CC JG NB.

References

1. Costanza R, Kemp M, Boynton W (1993) Predictability, scale, and biodiversity in coastal and estuarine ecosystems: implications for management. Ambio 22: 88–96.
2. Harvell CD, Kim K, Burkholder JM, Colwell RR, Epstein PR, et al. (1999) Emerging marine diseases - Climate links and anthropogenic factors. Science 285: 1505–1510.
3. Nicholls RJ, Wong PP, Burkett VR, Codignotto JO, Hay JE, et al. (2007) Coastal systems and low-lying areas. In: ML. Parry, OF. Canziani, JP. Palutikof, PJ. van der Linden, CE. Hanson, eds. Climate Change 2007: Impacts, Adaptation and Vulnerability, Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge: Cambridge University Press. pp 315–356.
4. Dayton PK, Trush SF, Agardy TM, Hofman RJ (1995) Environmental effects of marine fishing. Aquatic conservation: Marine and Freshwater ecosystems 5: 205–232.
5. Jackson J, Kirby M, Berger W, Bjorndal KA, Botsford LW, et al. (2001) Historical overfishing and the recent collapse of coastal ecosystems. Science 293: 629–638.
6. Harvell C, Mitchell CE, Ward JR, Altizer S, Dobson A, Ostfeld R, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296: 2158–2162.
7. Steneck RS, Carlton JT (2001) Human alterations of marine communities: students beware! In: Bertness MD, Gaines SD, Hay ME, eds. Marine community ecology. Sunderland: Sinauer. pp 159–181.
8. Hughes TP, Baird AH, Bellwood DR, Card M, Connolly SR, et al. (2003) Climate change, human impacts, and the resilience of coral reefs. Science 301: 929–933.
9. Kappel CV (2005) Losing pieces of the puzzle: threats to marine, estuarine, and diadromous species. Frontiers in Ecology and the Environment 3: 275–282.
10. Harley CDG, Randall Hughes A, Hultgren KM, Miner BG, Sorte CJB, et al. (2006) The impacts of climate change in coastal marine systems. Ecology Letters 9: 228–241.
11. Hughes TP, Bellwood DR, Folke C, Steneck RS, Wilson J (2005) New paradigms for supporting the resilience of marine ecosystems. Trends in Ecology and Evolution 20: 380–386.
12. Walther GR, Post E, Convey P, Menzel A, Parmesan C, et al. (2002) Ecological responses to recent climate change. Nature 416: 389–395.
13. McClanahan TR, Ateweberham M, Muhando A, Maina J, Mohammed MS (2007) Effects of climate and seawater temperature variation on coral bleaching and mortality. Ecological Monographs 77(4): 503–525.
14. Romano J, Lugrezi M (2007) Série du marégraphe de Marseille: mesures de températures de surface de la mer de 1885 à 1967. Comptes Rendus Geosciences 339: 57–64.
15. Bensoussan N, Romano JC, Harmelin JG, Crisci C, Pascual J, et al. (2009) Warming trends, regional fingerprints and future trajectories of NW Mediterranean coastal waters. In: UNEP RAC/SPA (eds) (2009) Proceedings of the 1st Mediterranean symposium on the conservation of the coralligenous and other calcareous bio-concretions, Tabarka, Tunisia. pp 167–168.
16. Coma R, Ribes M, Serrano E, Jimenez E, Salat J, et al. (2009) Global warming-enhanced stratification and mass mortality events in the Mediterranean. Proceedings of the National Academy of Sciences 106: 6176–6181.
17. Vargas-Yáñez M, Moya F, García-Martínez MC, Tel E, Zunino P, et al. (2010) Climate change in the Western Mediterranean Sea 1900–2008. Journal of Marine Systems 82: 171–176.
18. Francour P, Boudouresque CF, Harmelin-Vivien M, Harmelin JG, Quignard JP (1994) Are the Mediterranéan waters becoming warmer? Information from biological indicators. Mar Pollut Bull 28: 523–526.
19. Bianchi CN (2007) Biodiversity issues for the forthcoming tropical Mediterranean Sea. Hydrobiologia 580: 7–21.
20. Pérez T, Garrabou J, Sartoretto S, Harmelin JG, Francour P, et al. (2000) Mortalité massive d'invertébrés marins: un évènement sans précédent en Méditerranée nord-occidentale. C R Acad Sc Paris, Sc Vie 323: 853–865.
21. Garrabou J, Coma R, Bensoussan N, Bally M, Chevaldonné P, et al. (2009) Mass mortality in Northwestern Mediterranean rocky benthic communities: effects of the 2003 heat wave. Global Change Biology 15: 1090–1103.
22. Bensoussan N, Romano JC, Harmelin JG, Garrabou J (2010) High resolution characterization of northwest Mediterranean coastal waters thermal regimes: To better understand responses of benthic communities to climate change. Estuarine, Coastal and Shelf Science 87: 431–441.
23. Vezzulli L, Previati M, Pruzzo C, Marchese A, Bourne DG, et al. (2010) Vibrio infections triggering mass mortality events in a warming Mediterranean Sea. Environmental Microbiology 12: 2007–2019.
24. Huete-Stauffer C, Vielmini I, Palma M, Navone A, Panzalis P, et al. (2011) Paramuricea clavata (Anthozoa, Octocorallia) loss in the Marine Protected Area of Tavolara (Sardinia, Italy) due to a mass mortality event. Marine Ecology 32: 107–116.
25. Cerrano C, Bavestrello G, Bianchi CN, Cattaneo-Vietti R, Bava S, et al. (2000) Catastrophic Mass-mortality Episode of Gorgonias and Other Organisms in the Ligurian Sea (North-western Mediterranean), Summer 1999. Ecology Letters 3: 284–293.
26. Linares C, Coma R, Diaz D, Zabala M, Hereu B, et al. (2005) Immediate and delayed effects of a mass mortality event on gorgonian population dynamics and benthic community structure in the NW Mediterranean Sea. Marine Ecology Progress Series 305: 127–137.
27. Garrabou J, Perez T, Sartoretto S, Harmelin JG (2001) Mass mortality event in red coral (Corallium rubrum, Cnidaria, Anthozoa, Octocorallia) population in the Provence region (France, NW Mediterranean). Marine Ecology Progress Series 217: 263–272.
28. Margalef R (1985) The Western Mediterranean. London: Pergamon Press.
29. Gili JM, Ballesteros E (1991) Structure of cnidarian populations in Mediterranean sublitoral benthic communities as a result of adaptation to different environmental conditions. Oecologia Aquatica 10: 243–254.
30. True MA (1970) Étude quantitative de quatre peuplements sciaphiles sur substrat rocheux dansla région marseillaise. Bulletin de l'Institut Oceanographique Monaco 1410: 1–48.
31. Harmelin JG (2004) Environnement thermique du benthos côtier de l'île de PortCros (parc national, France, Méditerranée nord-occidentale) et implications biogéographiques. Scientific Reports Port-Cros national park, Fr 20: 173–194.
32. Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica 4(1): 9.

33. Schär C, Vidale PL, Lüthi D, Frei C, Häberli C, et al. (2004) The role of increasing temperature variability in European summer heat waves. Nature 427: 332–336.
34. Rebetez M, Dupont O, Giroud M (2009) An analysis of the July 2006 heatwave extent in Europe compared to the record year of 2003. Theoretical and Applied Climatology 95: 1–7.
35. Millot C (1979) Wind induced upwellings in the Gulf of Lions. Oceanol Acta 2: 261–274.
36. Coma R, Ribes M (2003) Seasonal energetic constraints in Mediterranean benthic suspension feeders: effects at different levels of ecological organization. Oikos 101: 205–215.
37. Bally M, Garrabou J (2007) Thermodependent bacterial pathogens and mass mortalities in temperate benthic communities: a new case of emerging disease linked to climate change. Global Change Biology 13: 2078–2088.
38. Torrents O, Tambutté E, Caminiti N, Garrabou J (2008) Upper thermal thresholds of shallow vs. deep populations of the precious Mediterranean red coral Corallium rubrum (L.): Assessing the potential effects of warming in the NW Mediterranean. Journal of Experimental Marine Biology and Ecology 357: 7–19.
39. Previati M, Scinto A, Cerrano C, Osinga R (2010) Oxygen consumption in Mediterranean octocorals under different temperatures. Journal of Experimental Marine Biology and Ecology 390: 39–48.
40. Coles S (1975) A comparison of effects of elevated temperature versus temperature fluctuations on reef corals at Kahe Point, Oahu. Pacific Sciences 29: 15–18.
41. Sammarco PW, Winter A, Stewart JC (2006) Coefficient of variation of sea surface temperature (SST) as an indicator of coral bleaching. Marine Biology 149: 1337–1344.
42. Wilson RS, Franklin CE (2002) Testing the beneficial acclimation hypothesis. Trends in Ecology and Evolution 17: 66–70.
43. Williams G (1966) Adaptation and Natural Selection. Princeton: Princeton University Press.
44. Kawecki TJ, Ebert D (2004) Conceptual issues in local adaptation. Ecology Letters 7.
45. Hendry AP, Kinnison MT (1999) The pace of modern life: measuring rates of contemporary microevolution. Evolution 53: 1637–1653.
46. Baums IB (2008) A restoration genetics guide for coral reef conservation. Molecular Ecology 17: 2796–2811.
47. Smith-Keune C, Oppen M (2006) Genetic structure of a reef-building coral from thermally distinct environments on the Great Barrier Reef. Coral Reefs 25: 493–502.
48. Oliver TA, Palumbi SR (2011) Do fluctuating temperature environments elevate coral thermal tolerance? Coral Reefs.
49. Duran S, Giribet G, Turon X (2004) Phylogeographical history of the sponge Crambe crambe (porifera, poecilosclerida): range expansion and recent invasion of the Macaronesian islands from the Mediterranean Sea. Molecular Ecology 13: 109–122.
50. Ledoux JB, Mokhtar-JamaÏ K, Roby C, Feral JP, Garrabou J, et al. (2010) Genetic survey of shallow populations of the Mediterranean red coral [Corallium rubrum(Linnaeus, 1758)]: new insights into evolutionary processes shaping nuclear diversity and implications for conservation. Molecular Ecology 19: 675–690.
51. Antonov J, Levitus S, Boyer TP, Conkright M, O'Brien T, Stephens C (1998) World Ocean Atlas 1998 Vol. 1. In: Temperature of the Atlantic Ocean, NOAA Atlas NESDIS, 27. 166 p.
52. Zabala M, Ballesteros E (1989) Surface-dependent strategies and energy flux in benthic marine communities or, why corals do not exist in the Mediterranean. Scientia Marina 53: 3–17.
53. Garrabou J, Ballesteros E, Zabala M (2002) Structure and Dynamics of North-western Mediterranean Rocky Benthic Communities along a Depth Gradient. Estuarine, Coastal and Shelf Science 55: 493–508.
54. Somot S, Sevault F, Déqué M, Crépon M (2008) 21st century climate change scenario for the Mediterranean using a coupled atmosphere-ocean regional climate model. Global and Planetary Change 63: 112–126.
55. Coll M, Piroddi C, Steenbeek J, Kaschner K, Ben Rais Lasram F, et al. (2010) The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLoS ONE 5: e11842.
56. Rodolfo-Metalpa R, Richard C, Allemand D, Ferrier-Pages C (2006) Growth and photosynthesis of two Mediterranean corals, Cladocora caespitosa and Oculina patagonica, under normal and elevated temperatures. Journal of Experimental Biology 209: 4546–4556.
57. Ferrier-Pages C, Tambutte E, Zamoum T, Segonds N, Merle PL, et al. (2009) Physiological response of the symbiotic gorgonian Eunicella singularis to a long-term temperature increase. Journal of Experimental Biology 212: 3007–3015.

Annexe 2

Complementary information of Chapter 3: field T regimes of the studied regions, complementary experiments results and aquariums food concentration

In Chapter 5 principal results of thermotolerance experiments carried out during 2009 (March to November) were analyzed. Here, we provide information of field T regimes of the studied regions and results from experiments which results were not presented in the chapter. Also supplementary data of food concentration in the aquariums is provided.

Field T data of the studied regions

Table 1. T descriptors of the three studied regions at 20 and 40 m depth. Statistics were calculated for the summer period (1st July – 30 September) and for all the years where hourly T records were available. For the Mean T, values of this statistic for all the years were averaged (±SD), while for all other statistics, the rank (minimum and maximum values) is presented. Max T: maximum T, CV: coefficient of variation, % t ≥ 21, 22, 23, 24, and 25: percentage of summer time above different T thresholds from 21 to 25°C.

Region	Period	Depth (m)	MeanT (°C)	Max. T (°C)	CV (%)	% t ≥ 21°C	% t ≥ 22°C	% t ≥ 23°C	% t ≥ 24°C	% t ≥ 25°C
Medes	2002-2010	20	20.1 ± 0.8	21.9 - 25.1	6.3 - 15.0	4.1 - 50.0	0.0 - 37.8	0.0 - 24.9	0.0 - 8.8	0 - 0.3
Marseille	1999-2010		18.0 ± 0.7	22.1 - 25.4	12.0 - 18.5	3.3 - 41.7	0.3 - 39.9	0.0 - 26.7	0.0 - 4.9	0.0 - 0.4
Scandola	2004-2010		21.2 ± 0.8	24.2 - 26.9	5.9 - 11.1	33.0 - 77.2	21.8 - 67.5	3.2 - 51.7	0.4 - 18.7	0 - 4.5
Medes	2002-2010	40	16.8 ± 0.6	22.5 - 24.9	12.5 - 17.1	6.2 - 10.8	0.7 - 7.7	0.0 - 5.1	0.0 - 1.5	0.0
Marseille	2000-2010		15.8 ± 0.4	19.7 - 23.6	6.3 - 15.7	0.0-14.1	0.0 - 8.5	0.0 - 1.1	0.0	0.0
Scandola	2004-2010		16.4 ± 0.9	20.7 - 23.0	6.3 - 8.9	0.0 - 2.3	0-0.5	0.0	0.0	0.0

Supplementary experiments

Table 2. Principal characteristics of supplementary experiments. R_sh: Riou shallow, Ph_sh: Pharillons shallow, GP_sh: Grotte Pérès shallow, M_sh: Medes shallow, M_d: Medes deep.

	T (oC)	Sample date	Stabulation (days)	Exp. dates	Dur. (days)	Num. col. (C/T)	Site
1	26±0.2	11 March	106	25/06-12/07	18	36/27	R_sh, Ph, sh, GP_sh
2	26±0.5	3 September	4	7/09-6/10	30	10/15	M_sh
3	25±0.7	8 October	3	11/10-1/11	22	60/60	M_sh, M_d
4	27±1	22 October	14	5/11-9/11	5	*/60	M_sh, M_d

*no controls were available for this experimental set

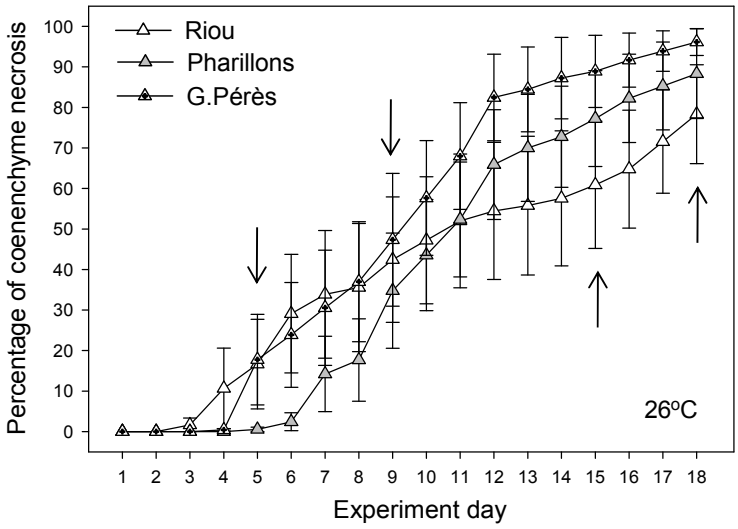

Figure 1. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from shallow (20 m) populations of Marseille submitted to 26°C. Colonies were collected in March. PERMANOVA analysis (Factor Site: random, Factor Time: fixed) did not show significant differences among populations coenenchyme necrosis (p-value >0.05).

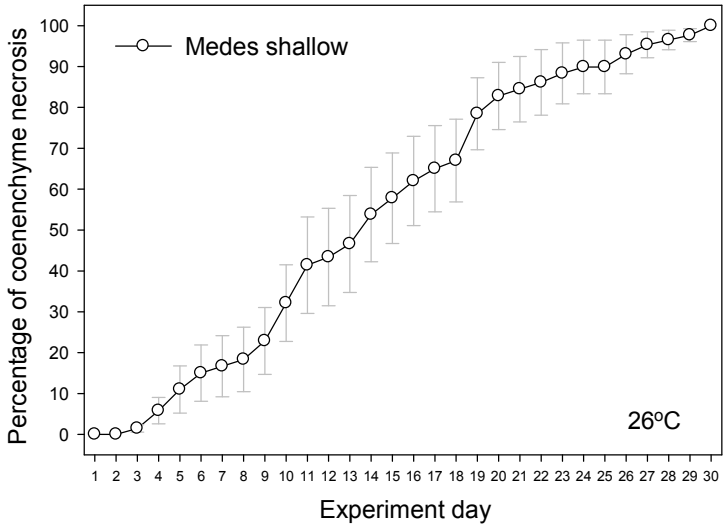

Figure 2. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from a shallow (20 m) population of Medes submitted to 26°C. Colonies were collected in September.

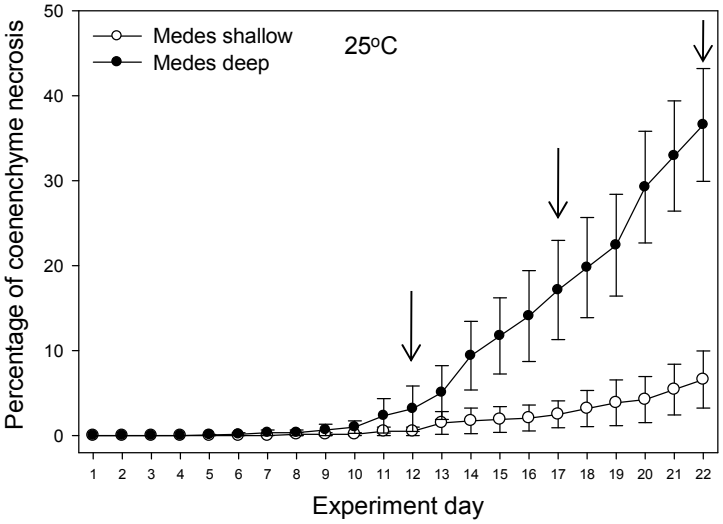

Figure 3.. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from shallow (20 m) and deep (40 m) populations of Medes submitted to 25°C. Colonies were collected in October. PERMANOVA analyses (Factor Depth and Time fixed) attested for significant differences of coenenchyme necrosis curves (p-value <0.001).

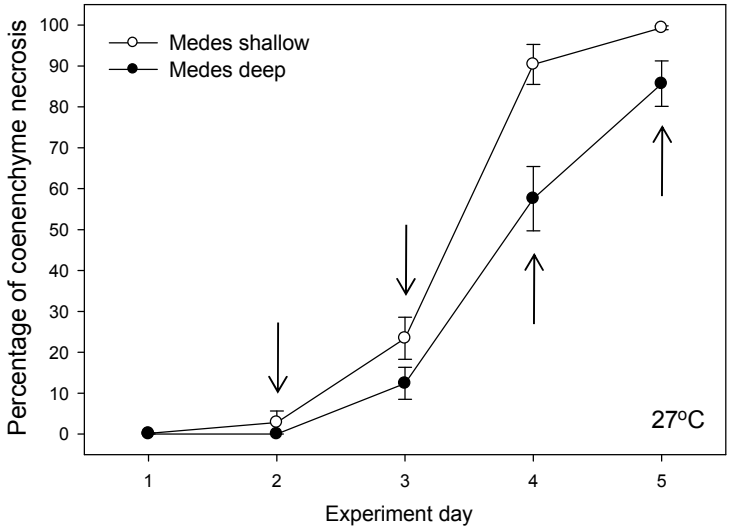

Figure 4. Mean (±SE) percentage of coenenchyme necrosis of *P. clavata* colonies from shallow (20 m) and deep (40 m) populations of Medes submitted to 27°C. Colonies were collected in October. PERMANOVA analyses (Factor Depth and Time fixed) attested for significant differences of coenenchyme necrosis curves between depths (p-value <0.001).

Supplementary data of food concentration in the aquariums

Table 3. Basal and after feeding events food concentration (average ± SD) in the Preliminary and Inter-regional/inter-depth experiments. Basal concentrations were measured before every feeding event (two times per week during all the experiments long). For the after feeding events concentrations, measures were taken at the moment of the food supply and at every hour during five hours after food supply (six measures) once at each experiment (dates of Chl-a and POC – zooplankton samplings are indicated). Food concentration was calculated integrating the values of the 5 hours. In the preliminary experiment, measures were taken for the Controls and Treatments buffers tanks (where food was supplied) while in the Inter-regional/inter-depth experiment measures were obtained from one of the Treatments and one the Controls tanks. The grater concentrations of food observed in the Preliminary experiment can be explained by the smaller volume of the Preliminary experiments tanks and the lower number of colonies within them (same concentration of food was provided for both experiments), but also by the different moment of food sampling, considering that higher food concentrations are found in the field during late winter than in summer (Coma and Ribes, 2003). Chl-a: chlorophyll a, POC: Particulate Organic Carbon.

	Basal concentrations			After feeding event concentration		
	Num. of samples	**Prelim. (March)**	**Inter-reg./ inter depth (June)**	**Num. of samples**	**Prelim. (24 March – 9 April)**	**Inter-reg./ inter depth (14 July – 17 July)**
[Chl-a] (ug/l)	14	0.13 ± 0.06	0.03 ± 0.02	12 (2 per hour during six hours)	8.00 ± 0.94	2.31 ± 0.25
[POC] (ug/l)	14	0.43 ± 0.13	0.31 ± 0.04	12	903 ± 44	527 ± 73
[Naunplii] (organisms/l)	14	3.75 ± 15	0.00 ± 0.00	12	788 ± 158	415 ± 72

Supervised Machine Learning algorithms and their applications to ecological data: a review

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Abstract

In this paper we present a general overview of supervised Machine Learning (ML) algorithms as well as a brief survey of the "state of the art" about the applications of these techniques to the particular field of ecological data. In the first part of the paper we describe in a conceptual way the general framework of ML and explain the basis of the underlying theory. In the second part we present some outstanding ML techniques to treat ecological data. Finally, in the third part we present a practical example that can be potentially handled with the different proposed techniques. An extensive list of references is given in order to allow the reader to go further and deeper on the discussed topics.

Keywords: Machine Learning, Ecological Data, Regression Analysis, Classification Rules, prediction, feature selection.

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Annexe 4

Complementary information of Chapter 3: R scritp – Bagging for regression

```
bagging.reg <-
function (formula, data, mfinal = 100, minsplit = 5, cp = 0.01, maxdepth = nlevels(vardep))
{
   formula <- as.formula(formula)
   vardep <- data[, as.character(formula[[2]])]
   n <- length(data[, 1])
#   nclases <- nlevels(vardep)
   arboles <- list()
   replicas <- array(0, c(n, mfinal))
   for (m in 1:mfinal) {
      boostrap <- sample(1:n, replace = TRUE)
      fit <- rpart(formula, data = data[boostrap, ], minsplit = minsplit,
         cp = cp, maxdepth = maxdepth)
#       fleam <- predict(fit, newdata = data, type = "class")
      fleam <- predict(fit, newdata = data)

     ind <- as.numeric(vardep != fleam)
      err <- sum(ind)/n
      arboles[[m]] <- fit
      replicas[, m] <- boostrap
      if (m == 1) {
         acum <- summary(fit$fram[, 1])
      }
      else {
         acum1 <- summary(fit$fram[, 1])
         acum <- acum + acum1
      }
   }
   pred <- 0
   for (m in 1:mfinal) pred <- pred + predict(arboles[[m]], data)
   pred = pred / mfinal
   acum <- acum[-1]/sum(acum[-1]) * 100
   ans <- list(formula = formula, trees = arboles, class = pred, samples = replicas, importance = acum)
   class(ans) <- "bagging.reg"
   ans
}

predict.bagging.reg <-
function (object, newdata, ...)
{
   vardep <- newdata[, as.character(object[[1]][[2]])]
   mfinal <- length(object[[2]])
   n <- length(newdata[, 1])
   pred <- 0
   for (m in 1:mfinal) pred <- pred + predict(object[[2]][[m]], newdata)
      pred = pred /mfinal
   error = mean((pred- vardep)^2)
   output <- list(class = pred, error = error)
}
```

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